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. . . *THROUGH CHEMISTRY*

ORAN CECIL BOYD, 1894-1952

Theodore T. Kozlowski

On January 5, 1952, Oran Cecil Boyd died at his home in Amherst at the age of 57. Born on a farm in Diamond, Missouri, on March 16, 1894, the son of David D. and Agnes (Miller) Boyd, he graduated from Hooker Oklahoma High School and received the A.B. degree in 1916 from Oklahoma Agricultural and Mechanical College. He became graduate assistant in botany and plant pathology at the University of Missouri, 1916-17, but interrupted his studies to enlist in the United States Army at Guymon, Oklahoma, September 17, 1917. He was discharged as a second lieutenant January 10, 1919, and returned to the University of Missouri where he received his M.A. degree in 1920.

The next 3 years were spent as a graduate student and industrial fellow in the Department of Plant Pathology, Cornell University. During this time he did field work in the summers with the Suffolk County (Long Island) Potato Growers Association. After receiving his Ph.D. from Cornell in September 1923, Dr. Boyd became plant pathologist of the Georgia State Board of Entomology stationed at Thomasville and Albany, Georgia, until 1929. Here he was in charge of all disease control projects undertaken by the Board, including inspection, diagnosis and quarantine work, and carried on many research projects on vegetable diseases.

In January 1930 he came to the University of Massachusetts. In his capacity as extension specialist in plant pathology Dr. Boyd rendered the highest order of service to the State of Massachusetts. Associates will always remember him for his tremendous knowledge of his subject, his keen observation and unusual ability to diagnose diseases and abnormalities in the field. He was willing always to do more than his share.

His loyalty, his balanced judgment in all matters, and his complete modesty won for him the admiration of all who knew or worked with him.

The growers of Massachusetts learned early that Dr. Boyd was a sympathetic counselor who was there to help them. The rapport and mutual confidence established between them was the source of significant progress in disease control among the plant industries of Massachusetts. The growers knew that Dr. Boyd understood their problems thoroughly and could provide practical suggestions. Under his leadership the growers and agents learned to help themselves. His was a rare combination of highly technical yet practical gifts, and he had unusual ability both to impart instruction and to arouse the enthusiasms of all groups of men. Beyond everything else, to the growers he served so well "Doc" Boyd was a real friend.

Dr. Boyd was always a student. Gifted with a keen mind, an amazing capacity for work, and a strong desire to learn and to help others to learn, he methodically kept abreast of his science. Even his home garden became the trial ground for new ideas and methods; no produce could be gathered until "records" were taken.

ORAN CECIL BOYD
1894-1952

Dr. Boyd's scientific interests covered a wide range. Although primarily a vegetable pathologist he published papers dealing with fruit diseases, tobacco, and trees. His Massachusetts Extension Service publications, numbering more than 100, were in wide demand throughout the country.

Dr. Boyd was a member of Alpha Zeta, Phi Kappa Phi, Kappa Sigma, Gamma Alpha, Sigma Xi, Epsilon Sigma Phi, The American Phytopathological Society and The American Association for the Advancement of Science. He was also a member of Grace Episcopal Church, Amherst, and the Amherst Post 148 American Legion.

He is survived by his wife, Nana Perry (Burwell) whom he married at Ithaca, New York, March 27, 1921; three sons, Oran Cecil, Jr. of Santa Barbara, California; Dallas David, Georgetown, Conn.; and Harold Eugene, Levittown, New York.

Titles of a few of Dr. Boyd's publications follow:

Control measures for watermelon diseases. Georgia State Board of Entomology Circ. 38. 1924.

The relative efficiency of some copper dusts and sprays in the control of potato diseases and insect pests. New York (Cornell) Agr. Exp. Sta. Bull. 451. 1926.

Report on the experiment of 1925 for pecan scab control at Baconton, Georgia. (Abstr.) Phytopathology 16: 644-645. 1926.

Comparison of various disinfectants in the treatment of sweet potatoes for black-rot control: A progress report. (Abstr.) Phytopathology 18: 153-154. 1928.

A bacterial disease of tung-oil tree. Phytopathology 20: 756-757. 1930.

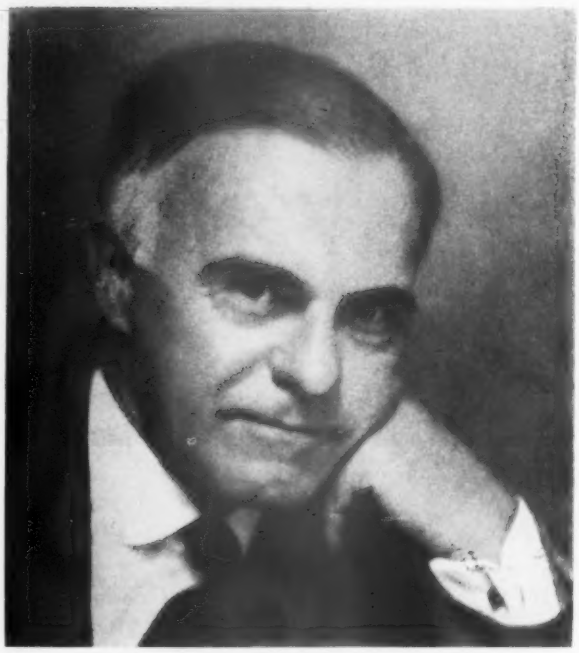
Disease control in the home vegetable garden. Mass. Ext. Serv. Feb. 16, 1934.

Evidence of seed-borne nature of late blight (*Phytophthora infestans*) of tomatoes. (Abstr.) Phytopathology 25: 7. 1935.

The cause, appearance and seasonal habits of garden diseases. Mass. Ext. Serv. March 1937.

HERBERT SPENCER JACKSON, 1883-1951

D. L. Bailey



HERBERT SPENCER JACKSON
1883 — 1951

With the death of Professor Herbert Spencer Jackson, which occurred suddenly at his home in Toronto, Ontario on December 14th, 1951, as a result of coronary thrombosis, American botanists lost a distinguished colleague and the field of systematic mycology one of its great men.

Born at Augusta Centre, N. Y., in 1883, Dr. Jackson later attended Cornell, where he received the A.B. degree in 1905. While at Cornell, he came under the encouraging influence of Professor G. F. Atkinson. There also began a friendship with Professor H. H. Whetzel, which grew through the years, until the latter's death in 1944. Together they collected intensively and widely in the Ithaca regions.

After leaving Cornell, Dr. Jackson went to the Delaware Experiment Station where he stayed until 1908. As the Austin Teaching Fellow in Botany, he studied mycology with Farlow and Thaxter at Harvard University, during 1908-09. He then accepted the position of Professor of Botany and Plant Pathology at the Oregon Agricultural College and held this post until 1915, when he went to the Purdue University Agricultural Experiment Station as Chief-in-Botany. He remained until 1928 and from 1915-28 held an appointment also as Agent, Bureau of Plant Industry, U. S. Department of Agriculture. In 1928 he went to the University of Toronto as Professor of Mycology in the Department of Botany and from 1941 until his death was head of that department.

Throughout his career, Professor Jackson's interests were essentially mycological and, within that field, his chief interests were taxonomy and phylogeny. While at Purdue he specialized on smuts and rusts and became recognized as an international authority on the taxonomy of these groups. His extended treatise on "Present Evolutionary Tendencies and the Origin of Life Cycles in the Uredinales," though published 20 years ago, still remains a classic. After coming to Toronto in 1929 Dr. Jackson examined and reported on the South American rusts in the Holway collections. Finding very few rusts among the local flora that were either new or basically interesting, he gradually shifted his emphasis to the Thelephoraceae, a neglected group much in need of taxonomic study, and replete with species of challenging interest. To a better understanding of this group, Professor Jackson and his students contributed some 16 journal articles. Dr. Jackson was also largely instrumental in building up, by critical collection and exchange, the fungus herbarium in his department, until it numbered approximately 94,000 accessions and included an unusually complete collection of the fungi occurring in a unique northern region, the Timagami Forest Reserve. He was singularly successful in the teaching and training of graduate students and attracted many outstanding students from other universities who studied under his direction for advanced degrees.

Dr. Jackson was a Fellow of the Royal Society of Canada and in 1944 was President of its section of Biological and Medical Sciences. He was a life-member of the American Phytopathological Society and President, in 1915, of its Pacific Division. In 1934 he was President of the Mycological Society of America. He was an Honorary member of the Canadian Phytopathological Society, a Fellow of the American Association for the Advancement of Science and his name was starred in the American Men of Science. He belonged to Sigma Xi, Gamma Alpha, and Phi Sigma fraternities.

Although modest and retiring, Professor Jackson was a man of ready understanding and broad sympathy with whom friendship developed slowly but was an exceedingly rewarding experience. He will be mourned universally as a scholar and a stalwart of his science, and deeply and lastingly missed by all who called him friend. He is survived by his widow, Edythe Doyle Jackson, whom he married in 1907; by a daughter, Mrs. C. D. Barrett of Imlay City, Michigan; a son, Dr. K. K. Jackson of Ithaca, N. Y., and a sister, Miss Charlotte Jackson, of Lansing Michigan, for many years a member of the library staff at the University there.

Dr. Jackson was author of more than 50 papers, the listing of which must be omitted for lack of space.

EFFECT OF TEMPERATURE ON THE SEVERITY OF FUSARIUM BASAL ROT IN NARCISSUS [1]

W. D. McClellan [2]

Basal rot of narcissus bulbs (caused by *Fusarium oxysporum* f. *narcissi* Snyder & Hansen) is the most serious disease of narcissus in the light soils of the warm coastal areas of eastern United States. Losses in bulb growing sections of Long Island and eastern North Carolina are often as much as 50 per cent and occasionally greater. Many varieties cannot be grown commercially in these sections because of basal rot. On the other hand, losses due to this disease in the commercial bulb producing areas of the Pacific Northwest seldom exceed 5 percent. It has been generally considered that basal rot is more severe in the East because of higher soil temperatures in early summer before digging. McWhorter and Weiss (5) stated that basal rot infection is dependent upon a moderately warm temperature (about 70° F.) and if the soil temperature averages as high as 75° to 80° F. the bulbs are likely to rot completely in the ground, but when the soil temperature remains as low as 60° during the maturing period the roots remain healthy. No data are given to support these conclusions. Gregory (1) showed that rotting of inoculated narcissus bulb scales was more rapid at 77° F. than at 62.6° and that losses in a naturally infected stock were about twice as great at 68° to 77° as at 50° to 53.6°. Hawker (3) showed that losses due to basal rot averaged 22.7 per cent when bulbs were stored at 80.6° compared with 7.5 per cent when similar lots were stored in a cool cellar where the temperature remained between 59° and 64.4° F. Infection takes place through roots and basal plate in early summer just before bulbs are dug (2, 4, 6). Since information on the effect of temperature on development of basal rot is fragmentary, tests were begun at Beltsville, Maryland, in 1947 to determine whether this disease has a minimum temperature requirement and, if so, whether the losses due to basal rot along the Atlantic seaboard could be reduced by using mulches late in the growing season to reduce soil temperatures.

EFFECT OF TEMPERATURE ON THE GROWTH OF FUSARIUM OXYSPORUM f. NARCISSI.—The effect of temperature on the growth of *Fusarium oxysporum* f. *narcissi* was determined by seeding plates of potato-dextrose agar with this organism. A small amount of agar on which the Fusarium was growing was placed in the center of each of 21 plates of agar. Three plates were placed in each of 7 boxes having controlled temperatures of 35°, 45°, 55°, 65°, 75°, 85°, and 95° F. Measurements of the diameter of the colonies were made daily until 1 plate or more were entirely covered with mycelium. Two tests were made and the average diameter of the colonies on 6 plates after 4 days at each temperature is shown in Figure 1. There was practically no growth at 35° or 45°, the growth rate increasing to a peak at 75° and then dropping off sharply. The plates incubated at 75° were covered with mycelium in 7 days.

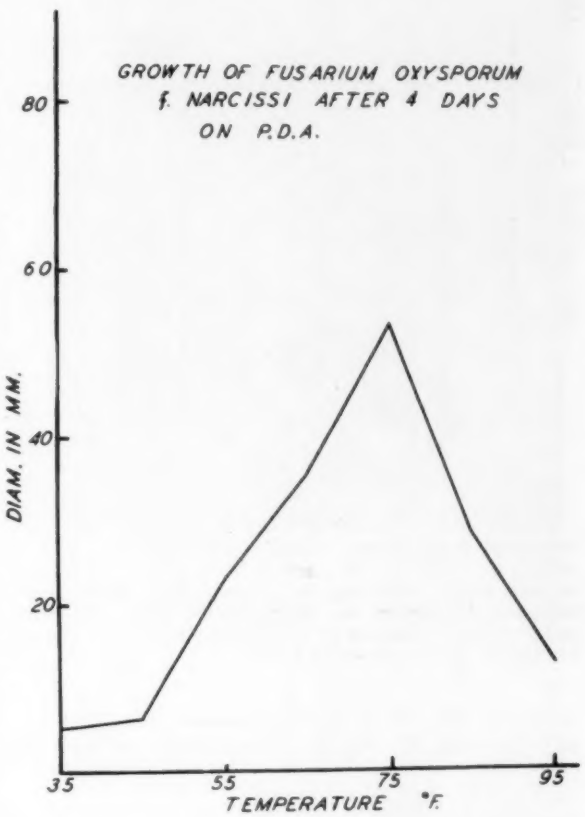


FIG. 1. Average diameter of mycelial colonies of *Fusarium oxysporum* f. *narcissi* after 4 days on potato-dextrose agar in petri dishes incubated at temperatures of 35°, 45°, 55°, 65°, 75°,85°, and 95°F.

EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF FUSARIUM ROT IN NARCISSUS ROOTS.—Precooled King Alfred narcissus bulbs were suspended in 400 cc. beakers so that their bases were immersed in water and then returned to 50° F. for 2 wk., until they had numerous good roots 25 to 60 mm. long. During this time the water was changed frequently to provide fairly adequate oxygen supply. The roots of 2/3 of these bulbs were then dipped in a spore and mycelial suspension of *Fusarium oxysporum* f. *narcissi*, those of the other 1/3 remained non-inoculated as a check. The bulbs were then returned to the beakers of water and 2 inoculated bulbs and 1 non-inoculated bulb were placed

[1] Accepted for publication April 2, 1952. Thanks are due Dr. C. J. Gould, Western Washington Experiment Station, who cooperated in supplying Washington-grown bulbs and who grew the Maryland bulbs in Washington.

[2] Formerly Pathologist, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture, Beltsville, Maryland.

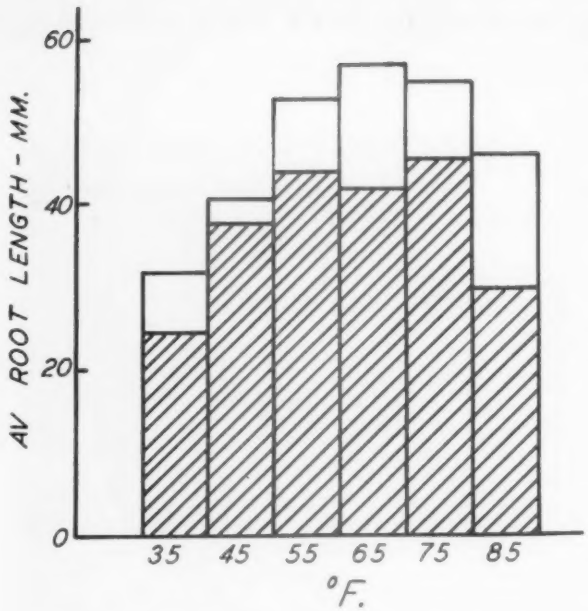


FIG. 2. Average length of roots of inoculated and non-inoculated King Alfred daffodils incubated for ten days at 35°, 45°, 55°, 65°, 75°, and 85° F., showing reduction of root growth following inoculation. The shaded areas represent the average root length of the inoculated roots, and the overall heights of the bar represent the average lengths of the non-inoculated roots.

at each of the following temperatures: 35°, 45°, 55°, 65°, 75°, and 85° F. The first evidence of basal rot was browning of the tips of inoculated roots after 4 days incubation at 85° F. At the end of approximately 10 days roots of the bulbs inoculated and incubated at 85° F. were brownish and flaccid, and the Fusarium had entered the bulb. Roots of the inoculated bulbs incubated at 75° were brownish and translucent, and the rot was just beginning to enter the bases of the bulbs. Smaller roots of the inoculated bulbs incubated at 65° were beginning to show signs of rot. Roots of all other bulbs, both inoculated and non-inoculated, were normal in appearance. However, inoculating the roots by dipping them in a spore and mycelial suspension caused a great reduction in root growth at all temperatures (Fig. 2). This reduction varied from 7.5 per cent at 45° to 35.2 per cent at 85° F.

EFFECT OF CONTROLLED SOIL TEMPERATURES ON BASAL ROT DEVELOPMENT.—Single nose King Alfred daffodils were inoculated and grown in modified Wisconsin-type soil temperature tanks in 3 experiments to gain information on the effect of soil temperature on development of Fusarium basal rot. Temperature tanks at 45°, 50°, 55°, 60°, 65°, and 70° F. were used. Each tank had 4 soil cans and 3 bulbs were planted per can. Bulbs in 3 of the 4 cans in each tank were inoculated by dipping the bases of the bulbs in a spore and mycelial suspension of *Fusarium oxysporum* f. *narcissi* just before planting. Bulbs in the fourth can were not inoculated. An air temperature of approximately 60° was maintained. All bulbs made rapid growth after planting because they had been precooled at 32° (Experiments I and II) or at 50° (Experiment III). Because there was a certain

TABLE 1.—*Effect of soil temperature on basal rot development in single nose King Alfred daffodils (data obtained from 3 experiments in which inoculated and uninoculated bulbs were planted in cans of soil in controlled temperature tanks)*

Treatment and soil temperature (°F.)	Number of plants with foliage symptoms I [a]	II [b]	III [c]	Severity of bulb rot I	II	Average healthy roots I number	II number	Average root length I cm.	II cm.
Noninoculated									
45	0/2 [d]	0/3	0/3	none	none	60.5	46.7	11.5	9.3
50	0/3	0/3	0/3	none	none	54.0	31.0	15.0	10.6
55	0/3	0/3	0/3	none	none	65.0	50.0	10.2	14.5
60	0/3	0/3	1/3	slight	none	68.0	28.3	12.3	13.9
65	1/2	0/3	2/3	severe	none	35.0	5.0	14.5	13.4
70	1/3	0/3	2/3	severe	none	29.0	1.0	18.8	12.9
Inoculated									
45	0/9	0/9	0/9	none	none	67.0	29.2	13.2	12.3
50	0/9	0/9	0/9	none	slight	47.1	14.4	18.5	12.2
55	4/9	0/9	3/9	slight	severe	28.9	11.8	15.4	12.2
60	7/9	2/9	4/9	severe	severe	2.0	1.4	6.2	11.5
65	8/9	2/9	7/9	severe	severe	1.0	0.0	7.2	—
70	6/8	8/9	9/9	severe	severe	0.0	0.0	0.0	—

[a] Bulbs used in Experiment I had been precooled at 32° F. from September 1948 to January 4, 1949. The data were obtained 45 days after planting.

[b] Bulbs used in Experiment II had been precooled at 32° F. from September 1948 to February 21, 1949. The data were obtained 28 days after planting.

[c] Bulbs used in Experiment III had been precooled at 50° F. from October 16 to December 20, 1950. Data were obtained 68 days after planting.

[d] Number of plants with symptoms number tested.

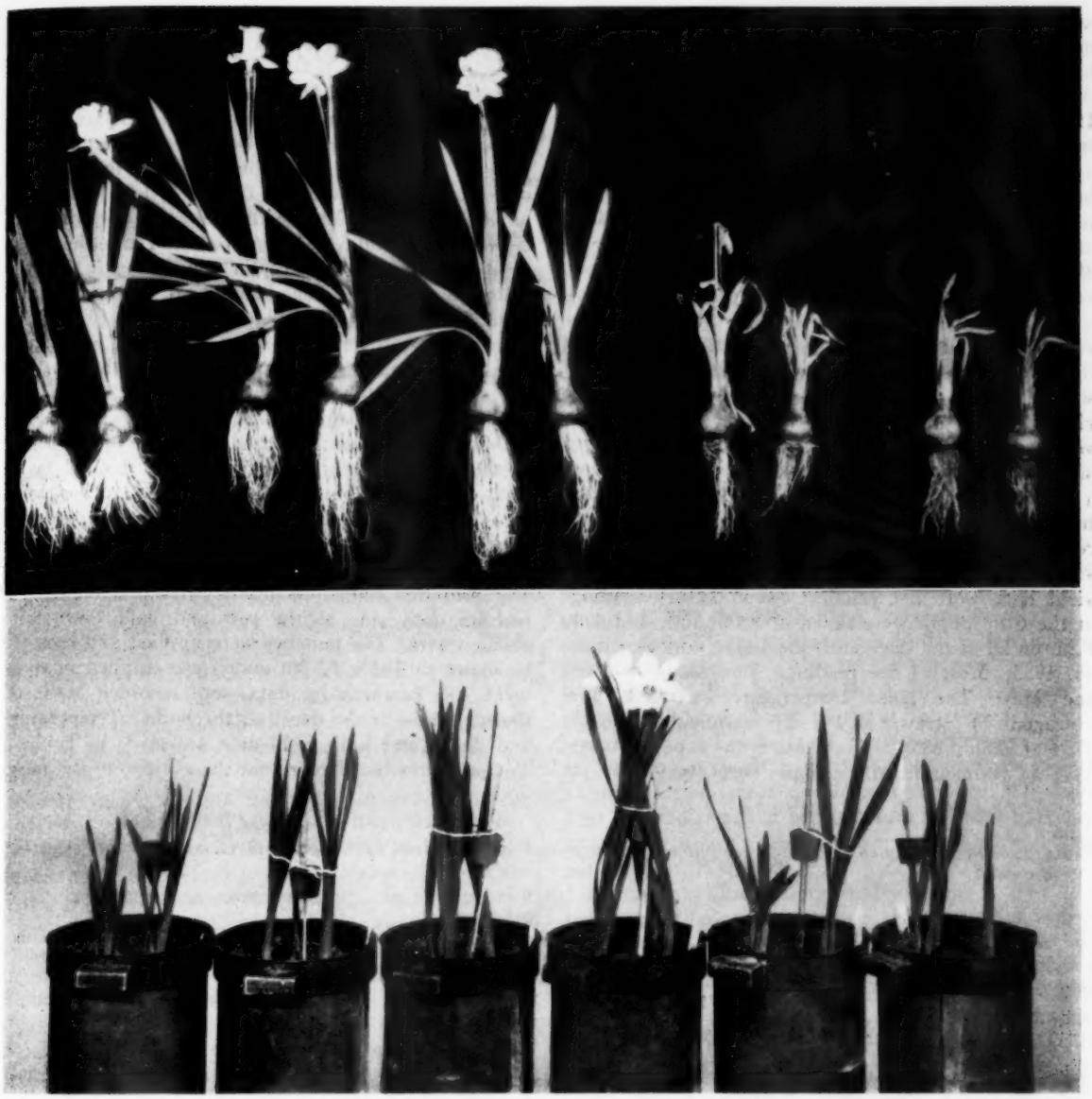


FIG. 3. King Alfred narcissus grown in soil temperature tanks adjusted to the following temperatures, left to right: 45°, 50°, 55°, 60°, 65° and 70° F. Plants inoculated with *Fusarium oxysporium* f. *narcissi*, above, and non-inoculated, below. The inoculated plants grown at 70° were too badly rotted to be shown. The inoculated plants were photographed 45 days after inoculation and planting and the non-inoculated plants were photographed 38 days after planting.

amount of rot in the stock of bulbs used and incipient infection could not be detected at time of planting some bulb rot occurred in non-inoculated checks, (Table 1). Inoculated and non-inoculated plants in Experiment I are shown in Figure 3. Optimum above-ground growth of non-inoculated bulbs occurred at soil temperatures of 55° and 60° F. Root production is also favored by these temperatures (Table 1). Growth and production of foliage and flowers were very poor at 70° and considerably better at 65°. Flower development was very slow at soil temperatures of 45° and 50°. At 70° inoculated bulbs showed foliage symptoms very soon after growth began. Symptoms were slower to develop at 60° and 65° but developed far more rapidly than at 55°, 50°, or 45°. In fact there seemed to be a rather sharp break between 60° and 55° in the rapidity with which the disease progressed (Fig. 3). At soil temperatures of 60° the rot progressed so rapidly that normal flowering usually did not take place, whereas at 55° foliage symptoms did not become serious until after flowering.

EFFECT OF MULCHES ON THE DEVELOPMENT OF BASAL

ROT IN THE FIELD.—Since basal rot develops much more rapidly at 60° than at 55° it was thought that late season mulches might reduce soil temperatures in the field sufficiently to affect basal rot development. If soil temperatures could be reduced by only 5° during the late spring or early summer before digging, a reduction in amount of loss due to basal rot might be expected. Preliminary trials were made on some miscellaneous King Alfred planting stock in the field at Beltsville, Maryland, in the spring and summer of 1950. Mulches were applied March 31 when the flowers were in bud. At this time the ground was partially frozen in the early morning. Straw, coarse vermiculite and leaf mold were used. The straw and leaf mold were held down with 2 in. mesh chicken wire and the vermiculite with ¼" mesh hardware cloth. In 2 other mulch treatments, straw and vermiculite were covered with building paper. A sixth treatment consisted of building paper only and a seventh treatment had no mulch. The mulches extended about 18 in. on each side of the row. Four replications were used but, because of the extreme variability in size and quality of the bulbs used, bulb yield data were not reliable. Temperature readings at the 6 in. depth were taken at 8:00 A.M. and 4:00 P.M. on 25 of the days until the bulbs were dug June 26, 1950. Most of the readings were made in April and May. The mean temperature on these days averaged 1) straw, 52.9°; 2) vermiculite, 53.3°; 3) leaf mold, 53.0°; 4) straw with a paper cover, 53°; 5) vermiculite with a paper cover, 53.9°; 6) paper cover, 55.2°; and 7) no mulch, 55.1° F. Thus, slightly lower temperatures occurred when mulches were used.

In the fall of 1950 a more extensive mulching trial was started. Twenty-five hundred single-nose King Alfred bulbs were planted October 2 in 50 12½ ft. plots of 50 bulbs each. These 50-bulb lots were of uniform size and averaged 6 lb. per lot. They were planted in 5 rows, 10 50-bulb plots per row with 4 ft. between plots and 6 ft. between rows. Ten mulch treatments were made in 5 replications using 5 different materials and 1 unmulched treatment. These materials were as follows: (A) Alumnium foil (0.0015 in. gauge dead soft temper foil), a strip 2 ft. wide being laid on the soil surface on each side of the row and the edges being held down with soil. (B) Coarse vermiculite, consisting of a 2-in. layer held down with hardware cloth in strips 2 ft. wide on each side of the row. (C) Straw, a layer 2 in. thick 2 ft. on each side of the row held down with chicken wire. (D) Thin glass wool, 20 mils in thickness applied in single layers 18 in. wide on each side of the row, the layers being held down with soil along the edges. (E) Unrotted hardwood leaves, placed 3 in. deep 2 feet on each side of the row and held down with chicken wire. The mulches were applied and removed as shown in Table 2. No mulch was applied in treatment 10. Temperature data were recorded with soil thermographs at the depth of the bulbs. Temperature and flower and bulb yield data are given in Table 2. Temperatures listed represent the average of the mean

TABLE 2.—*Effect of mulch treatments on soil temperatures and yields of King Alfred narcissus bulbs at Beltsville, Maryland*

Mulch treatment	Mulching period	Av. soil temperatures (1950-51) [a] Nov. 21 to Jan. 22 °F.	March 6 to April 22 °F.	May 8 to July 9 °F.	Flowers produced [b] number	Av. date of bloom [c]	Healthy bulbs [b] number	Weight of healthy bulbs [b] ounces	Bulbs with basal rot [b] per cent
1. Aluminum foil	November 7 to April 18	33.6	45.7		229	April 10	221	737	7.1
2. Vermiculite	November 7 to April 18	33.4	45.7		232	April 12	227	712	4.2
3. Straw	November 7 to April 18	36.4	45.5		232	April 12	208	728	11.5
4. Glass wool	November 7 to April 18	34.8	46.2		237	April 11	207	673	6.7
5. Leaves	April 18 to July 16			68.6	235	April 10	213	674	4.0
6. Straw	April 18 to July 16			68.8	237	April 10	221	693	4.7
7. Straw	May 18 to July 16				233	April 10	213	659	7.8
8. Aluminum foil	May 18 to July 16			70.7	240	April 10	228	733	3.8
9. Leaves	May 18 to July 16			68.2	241	April 10	223	721	4.7
10. Unmulched	.....	33.3	45.5	70.6	240	April 10	226	732	4.2

[a] These soil temperatures were obtained from soil themograph charts at the depth of the bulbs. Five thermographs, one in each of 5 different mulching treatments, were used.

[b] None of the differences between treatments are significant statistically.

[c] A difference of 1 day is significant.

TABLE 3.—*Bulb planting and harvest dates and mean monthly temperatures during the bulb growing season at Beltsville, Maryland, and Puyallup, Washington*

Season and place grown	Planting date	Harvest date	Mean air temperature (°F.) [a]						
			Sept.	Oct.	Apr.	May	June	July	Aug.
1947-48									
Maryland	Sept. 30	June 28	66.8	60.2	53.2	62.2	69.7	75.0	72.9
Washington	Oct. 9	Aug. 11	59.7	53.2	47.0	54.0	62.6	63.2	62.4
1949-50									
Maryland	Sept. 21	June 26	63.8	59.8	48.6	60.3	69.3	72.5	72.0
Washington	Sept. 20	July 13	60.5	49.6	47.8	52.8	60.6	63.8	64.7

[a] Mean air temperatures were obtained from U. S. Weather Bureau, Climatological Data. Volumes 34-37. 1947-1950. The values for Washington are those from the Tacoma Station, about 15 mi. from Puyallup.

temperatures at 6:00 A.M., noon, 6:00 P.M., and midnight, each day. Straw and glass wool mulches kept the soil slightly warmer in the winter and early spring than that in the unmulched plots or those mulched with aluminum or vermiculite. Vermiculite absorbed moisture so rapidly that it was ineffective as a mulch for controlling soil temperatures. Leaves and straw reduced soil temperature by about 2° per day during late spring and early summer. They also prevented extreme fluctuations in temperature. For instance on April 21 the unmulched soil fluctuated 22° from 43° to 65° F. during the day whereas the soil mulched with straw varied only 6° from 47° to 53° F. These soil temperature reductions in the late spring and early summer did not affect bulb yields or amount of basal rot; these data indicated that the late season mulches used were ineffective for reducing basal rot infections.

COMPARISON OF THE SEVERITY OF BASAL ROT IN NARCISSUS GROWN IN WASHINGTON AND MARYLAND.—In 1947 and again in 1949 C. J. Gould and the writer exchanged King Alfred narcissus bulbs in connection with some studies on mercury injury. In both years a stock of Maryland single-nose bulbs was divided and part shipped to Washington where they were planted in the field at the Western Washington Experiment Station at Puyallup and part planted in the field at the Plant Industry Station, Beltsville, Maryland. Similarly, lots of Washington bulbs were divided and part were planted in Washington and part at Beltsville. The planting and harvest dates are in Table 3, along with mean air temperatures at the 2 locations. Air temperatures were considerably higher at Beltsville than at Puyallup, particularly in early summer. Soil temperatures should show similar differences. As expected, the higher soil temperatures at Beltsville, therefore, resulted in increased basal rot severity (Table 4). Maryland-grown bulbs had 26.6 per cent rot in 1948 and 25.8 per cent in 1950 when grown in Maryland but when samples from these same lots of bulbs were grown in western Washington where the temperatures are lower only 5.1 and 6.7 per cent rot occurred. On the other hand, because of the lower temperatures, Washington-grown bulbs had a low incidence of basal rot (2.7 and 5.3 per cent); this increased only slightly when the bulbs were grown in Maryland (7.9 and 5.4 per cent in 1948 and 1949, respectively). Furthermore, the cooler temperatures of Washington were more favorable to bulb growth (Table 4) than the warm temperatures in Maryland. This is to be expected because data reported previously in this paper indicate greater growth at soil temperatures of 55° to 60° F.

DISCUSSION.—Laboratory studies indicate that if a reduction in soil temperature could be effected in late spring and early summer, losses due to basal rot would be greatly reduced. Attempts to bring about such a reduction in soil temperature through use of late season mulches did not reduce soil temperatures sufficiently to affect basal rot severity. On the other hand, basal rot losses in narcissus bulbs grown in areas such as western Washington, where the tem-

TABLE 4.—*Comparison of bulb yields and basal rot development in untreated King Alfred narcissus bulbs grown at Beltsville, Maryland, and Puyallup, Washington*

Season and source of bulbs	Where grown in season of trial	Bulbs planted number	Weight of bulbs planted ounces	Bulb data after storage		
				Healthy bulbs number	Weight of healthy bulbs ounces	Bulbs with basal rot per cent
1947-48						
Maryland	Maryland	200	111.0	94	99.0	26.6
	Washington	200	175.0	177	459.0	5.1
Washington	Maryland	200	373.0	186	396.0	7.9
	Washington	200	408.0	181	705.0	2.7
1949-50						
Maryland	Maryland	125	245.0	89	267.0	25.8
	Washington	125	300.0	116	329.5	6.7
Washington	Maryland	125	245.0	106	553.8	5.4
	Washington	125	290.0	108	541.9	5.3

peratures are cool during the growing season, are much lower than in bulbs grown in areas having hot summers such as Maryland, New York, and eastern North Carolina.

SUMMARY

The effect of temperature on the development of narcissus basal rot, caused by *Fusarium oxysporum* f. *narcissi* Snyder & Hansen, was investigated.

The optimum temperature for growth of the organism on agar cultures was 75° F. with very little growth at 35°, 45°, and 95° F.

Roots of precooled King Alfred narcissus bulbs inoculated with a spore and mycelial suspension of *F. oxysporum* f. *narcissi* showed evidence of infection 4 days after inoculation when incubated at 85° F. After 10 days at this temperature they were entirely rotted and the *Fusarium* had entered the bulb. Those incubated at 75° for 10 days were rotted almost as much, whereas those incubated at 65° were just beginning to show rot. Roots of bulbs incubated for 10 days at 35°, 45°, and 55° were not affected.

In temperature tanks in the greenhouse where soil temperatures of 45°, 50°, 55°, 60°, 65°, and 70° F. were maintained, rot developed most rapidly at 70° F. Symptoms were slower to develop at temperatures of 60° and 65° F. but they developed far more rapidly than at 55°, 50°, or 45°. Optimum growth of non-inoculated plants occurred with soil temperatures of 55° and 65° F.

Late season mulches with leaves, straw, and aluminum foil failed to reduce average soil temperatures enough to cause a reduction in severity of basal rot as measured by yields of healthy bulbs.

King Alfred narcissus bulbs grown at Beltsville, Maryland, in 1947 and 1949 had 26.6 and 25.8 per cent rot in the 2 respective years compared with 5.1 and 6.7 per cent rot in similar lots of bulbs shipped to Puyallup, Washington, and grown there. Washington-grown bulbs had a low incidence of basal rot (2.7 and 5.3 per cent, respectively, in the 2 yrs.) and this increased only slightly when similar lots of bulbs were grown in Maryland (7.9 and 5.4 per cent). Late spring and summer temperatures at Puyallup, Washington, average 8° to 12° lower than at Beltsville, and these lower temperatures are believed to account for the lower incidence of basal rot at Puyallup, Washington.

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MECHANICAL TRANSMISSION AND PROPERTIES OF ROSE MOSAIC VIRUS [1]

Robert W. Fulton

Virus diseases of rosaceous plants, with few exceptions, have been transmitted only by grafting or by means of insect vectors. Presumably the properties of these viruses, or of the plant extracts, limit transmissibility. Bawden and Kleczkowski (1) found that tannins in leaf extracts of strawberry precipitated the protein in the extracts. Such tannin-containing extracts also inactivated tobacco mosaic and tobacco necrosis viruses. Virus transmission thus might be prevented by the inactivating properties of the extract rather than by properties intrinsic to the virus.

A method of avoiding the detrimental effects of the extracts of certain hosts may be available if the virus can be transmitted to other hosts. Thus Moore, Boyle, and Keitt (8) transmitted necrotic ringspot virus from sour cherry to cucumber. In cucumber extract the virus was readily transmissible by mechanical methods to healthy cucumbers.

Transmission of rose mosaic virus to certain herbaceous hosts, on 1 of which, guar, it produced distinct local lesions, provided a starting point in determining factors affecting transmissibility. Some of these factors are described in the present paper, since they may be applicable to other viruses not now known to be transmissible mechanically.

MATERIALS AND METHODS.—Most of the plants used were grown from seed in a greenhouse at 80° F. Inoculations were made by wiping carborundum-dusted leaves with gauze pads dipped in virus-containing extracts, and the leaves were then immediately rinsed with distilled water. In determining the host range and properties of the virus, inoculum was taken from systemically infected trifoliate leaves of cowpeas (*Vigna sinensis* Endl. var. Blackeye) which had been infected recently. Dilutions were made by grinding known amounts of tissue in a volume of sodium phosphate buffer (pH 7.0) calculated to give the desired dilution. The tissue was punched from infected leaves with a 5/16 in. cork borer. The weight of 100 disks of tissue was approximately 1 gm. In making dilutions 1 disk was considered the equivalent of 0.01 ml. of extract. Thus 10 disks ground in 0.9 ml. of buffer constituted a 1:10 dilution. Assays were made by counting local lesions on the leaves of guar (*Cyamopsis tetragonalobus* (L.) Taub.).

IDENTITY OF THE VIRUS.—Naturally infected *Rosa setigera* Michx. leaves are shown in Figure 1, A. Symptoms varied from chlorotic ringspots and oakleaf patterns to isolated chlorotic spots. Unilateral involvement of the leaflets was not uncommon. The symptoms were very similar to those illustrated by Brierly and Smith (2) and referred to as rose mosaic. Leaves with the same types of symptoms are shown by Thomas and Massey (9), who refer to them as rose mosaics 2 and 3, making a distinction between different degrees of symptom severity. A number of descriptions (2, 7, 9, 10) agree with symptoms observed on rose in the present investigation and indicate the rather common occurrence of the disease. Two other virus diseases of rose seem sufficiently well differentiated from rose mosaic on the basis of symptoms. Rose wilt virus, described by Grieve (4) as being mechanically transmissible, induces recurving of leaves, abcission, and tip-killing. Grieve (5) concluded that rose wilt was the same as the disease described by Gigante (3), but distinct from rose mosaic or rose streak described by Brierly and Smith (2). The latter authors differentiated mosaic from streak on the basis of symptoms and cross protection tests.

TRANSMISSION.—The disease has been observed for several years on a few outdoor-grown *Rosa setigera* plants. There has been no spread to closely adjacent plants in 3 years. The virus was transmitted to cucumber and cowpea by macerating young rose leaves in buffer and inoculating the suspension to carborundum-dusted leaves or cotyledons. A total of 50 per cent of the cowpea plants became infected during May and June of 2 years, while less than 15 per cent of the cucumbers became infected. No transmission of the virus from rose was obtained after midsummer.

Infected cucumber seedlings showed faint chlorotic spots on inoculated cotyledons. The first leaf and the growing point soon became chlorotic, with subsequent necrosis and death of the plants within 3 wk. On cowpea chlorotic primary lesions were produced on the unifoliate leaves. Systemic invasion of the trifoliate leaves produced marked chlorotic rings, shown in Figure 1, D. The trifoliate leaves remained turgid for several days to a week and then became progressively more yellow and flaccid. Usually the leaves dropped and the plants died, but occasionally new leaves were retained and a few plants remained alive for several months.

In preliminary tests, using guar as a local lesion assay host, it was found that newly infected trifoliate leaves of cowpea provided reasonably infective inoculum. After the leaves became flaccid, however, the amount of virus obtained from them was markedly lower. Primary lesions on unifoliate cowpea leaves produced inoculum of only low infectivity.

HOST RANGE.—The plant species tested for susceptibility were grown from seed in most cases, and inoculated when young and vigorously growing. Although a pre-inoculation dark period did not increase susceptibility of guar, most of the other plants were darkened for 12 hr. before inoculation. Since lesions were produced on guar (Figure 1, E.) in about 3 days, inoculations were made to this plant to determine whether infection had been obtained. A number of infected species which showed slight or no symptoms also were tested by inoculation to cowpea.

The following species were artificially infected with rose mosaic virus:

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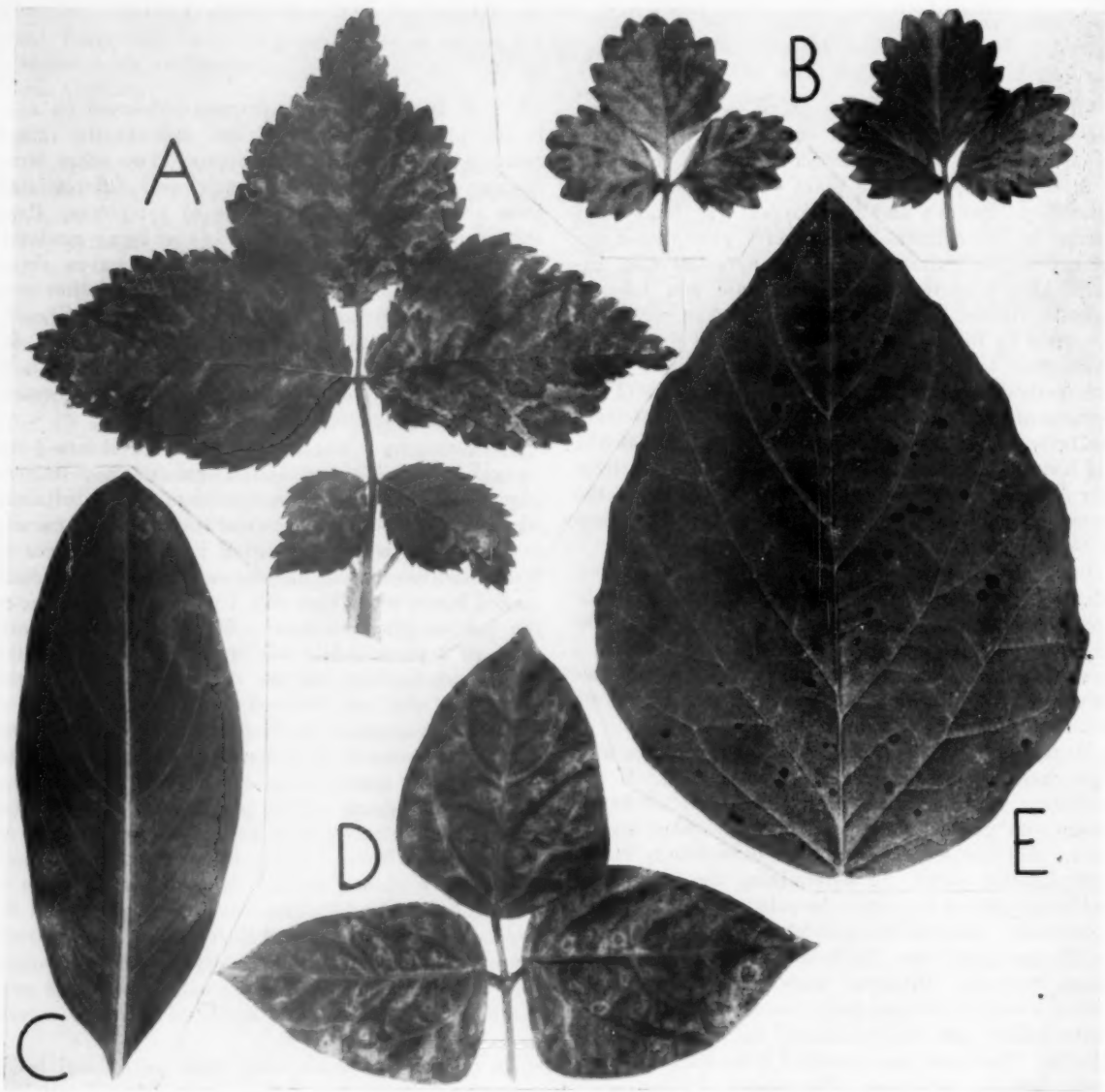

FIGURE 1. Symptoms of rose mosaic virus on (A) naturally infected *Rosa setigera*, and on mechanically inoculated (B) *Potentilla monspeliensis*, (C) *Vinca rosea*, (D) *Vigna sinensis*, and (E) *Cyamopsis tetragonalobus*.

APOCYNACEAE
Nerium oleander L.
Vinca rosea L.
CONVOLVULACEAE
Convolvulus tricolor L.
COMPOSITAE
Cynara cardunculus L.
CUCURBITACEAE
Citrullus vulgaris Schrad.
Cucumis sativus L.
Cucurbita maxima Duchesne.
C. pepo L.
LEGUMINOSAE
Crotalaria intermedia Kotschy
Cyamopsis tetragonalobus (L.) Taub.
Dolichos biflorus L.
Phaseolus aconitifolius Jacq.
P. aureus Roxb.
P. vulgaris L.
Vigna sinensis Endl.
ROSACEAE
Fragaria vesca L.
Fragaria sp. (Var. Marshall seedlings)
Potentilla monspeliensis L.
P. recta L.
Rosa multiflora Thunb.
R. setigera Michx.
SOLANACEAE
Nicotiana benthamiana Domin.
N. forgetiana Sand.
N. fragrans Hook.
N. glutinosa L.
N. occidentalis Wheeler
N. rustica L.
Petunia hybrida Vilm.

All but 3 of the 28 hosts listed were infected by mechanical inoculation. *Nerium oleander*, *Fragaria* sp. (strawberry), and *Rosa multiflora* were infected by grafting to infected *Vinca rosea*, *F. vesca*, and *R. setigera*, respectively. Chlorotic ringspot symptoms were produced on *Chenopodium album* L. but no virus could be recovered. A total of 92 other species, in 22 families, were not infected.

Despite numerous trials, *Rosa setigera* seedlings were infected on only 1 occasion by mechanical inoculation. Since leaf inoculations proved ineffective, roots were allowed to grow from the bottom of the pots into jars of water. These were rubbed with an infectious

extract of cowpea leaves. Three days later the plants were pruned to leave only about an inch of stem. New leaves on 1 plant showed the characteristic chlorotic ringspotting; the virus was transmitted from them to guar and cowpea mechanically, and by grafting to the rose variety "Better Times" and *Rosa multiflora*.

The symptoms, in the different species, consisted of chlorotic rings, mosaic (as on *Potentilla monspeliensis*, Figure 1, B), or indistinct brownish local lesions. Although prominent chlorotic ringspot symptoms were produced on *Vinca rosea*, as shown in Figure 1, C, this species was not seriously stunted. It yielded fairly infectious inoculum and was useful in maintaining a stock of the virus. On many of the hosts symptoms were very mild or absent, and the virus was recovered in only low concentrations. Of the solanaceous hosts, symptoms were produced only on *Nicotiana rustica*, which developed a mild systemic chlorotic spotting. *N. tabacum* L. and *Lycopersicon esculentum* Mill. remained healthy when grafted on infected *N. rustica*.

The virus was not transmitted by *Cuscuta campestris* Yuncker, *C. gronovii* Willd., or *C. subinclusa* Dur. and Hilg. from diseased *N. rustica* or from diseased *Vinca rosea*. In repeated trials *Myzus persicae* Sulz. failed to transmit the virus after having fed on infected *N. rustica*. Similarly, unidentified aphids found on naturally infected rose failed to transmit the virus to *Rosa setigera* seedlings or to *Fragaria vesca*.

FACTORS AFFECTING MECHANICAL TRANSMISSION.—It was found in preliminary trials that undiluted juice of infected cowpea leaves gave no infection on guar. In 3 subsequent trials, infected cowpea leaves ground with 24 parts of buffer gave an average of 202 lesions per leaf on guar. Juice pressed through gauze produced no lesions when inoculated undiluted, nor after being diluted 1:25 with buffer. Other trials showed that the addition of buffer to macerated tissue within 30 sec. after grinding failed to prevent complete inactivation. On the other hand inactivation was prevented when buffer or distilled water equal in amount to only ½ the weight of the tissue was added before grinding. Possibly some inactivator which has an irreversible effect on the virus is released when the leaves are ground. Presence of buffer during grinding may provide sufficient dilution so that only a part of the virus is affected.

No method, other than grinding the tissue in a diluent, was found for preventing the apparently immediate inactivation in undiluted extracts. The addition of buffering mixtures of phosphate salts, reducing agents, or various adsorbents to the tissue before grinding failed to maintain activity of the virus.

Although the addition of small amounts of distilled water to infected tissue before grinding prevented inactivation of the virus, more highly infectious extracts were obtained with 0.1 M phosphate buffer. In 3 trials to determine the effect of pH on infectivity, 1:25 extracts at pH 6.0, 7.0, and 8.0 gave averages of 16, 47, and 27 lesions, respectively, per leaf on guar. Neutral buffer was therefore used in nearly all transmission trials.

Of a number of buffers tried, none was superior to a mixture of sodium phosphates. One, a mixture of 0.05 M glycine and 0.05 M calcium chloride, gave a completely inactive extract. Other trials showed that it was the presence of the calcium ion that prevented infection. In trials with other salts tissue ground in neutral 0.05 M solutions of barium, magnesium, strontium, and sodium chlorides gave, respectively, averages of 0.3, 0.3, 1.8, and 46 lesions per half-leaf of guar. Evidently divalent metallic ions either inactivate the virus or in some way prevent infection.

Dilution trials were run by grinding 5/16 in. disks of infected cowpea tissue with a volume of buffer calculated to give the appropriate dilutions. Four dilutions were inoculated to half-leaves of guar in an incomplete block arrangement. The average numbers of lesions per half-leaf for 1:1, 1:5, 1:25, and 1:125 dilutions in 8 trials were 139, 129, 32, and 4, respectively. No infection was obtained, in other trials, at dilutions greater than 1:125.

AGING IN VITRO.—Aging trials were run on extracts prepared by grinding 1 part of infected cowpea tissue in 24 parts of buffer. These extracts lost most of their infectivity in 30 min. and were completely inactive after 60 min. Cysteine hydrochloride (0.01 M) added to the buffer had a pronounced preservative effect. In 4 trials the control, a 1:25 extract in buffer only, gave an average of 45 lesions per half-leaf when inoculated immediately after preparation, and no lesions after standing 1 hr. Similar extracts containing buffer plus 0.01 M cysteine hydrochloride gave averages of 38 lesions per half-leaf when inoculated immediately, and 25 lesions per half-leaf after 1 hr.

A similar preservative effect was found for 0.01 M sodium sulphite. When extracts were allowed to stand for 3 hr., however, those containing sodium sulphite were inactive, while those containing cysteine hydrochloride still retained at least a portion of their original infectivity.

When 1:25 extracts with and without 0.01 M cysteine hydrochloride were covered with mineral oil immediately after preparation the loss of virus activity occurred at approximately the same rate in each. Some infectivity was retained for 6 hr. but the extracts were inactive within 24 hr. It seemed evident, therefore, that oxidative effects were responsible for only part of the rapid aging of the virus *in vitro*. The protective effect of reducing agents slowed the rate of virus inactivation, but did not prevent the complete loss of virus activity within relatively short periods.

Attempts were made to preserve the virus by cutting infected tissue in small pieces and drying at 1° C. over a desiccant, as described by McKinney (6). The virus failed to survive in the tissue of cucumber, cowpea, *Vinca rosea*, or *Nicotiana rustica* in any trial. In other trials tissue was dried under partial vacuum at 1° C. over a desiccant, after flushing the container with oxygen-free air. No virus could be recovered from this tissue. In contrast, the viruses of tomato spotted wilt and potato yellow dwarf dried in this way

have remained active for 86 and 34 wk., respectively. It is evident that the virus of rose mosaic is even less stable than these 2 viruses.

THERMAL INACTIVATION POINT.—The thermal inactivation point of the virus was determined by heating disks of infected cowpea tissue in tubes with sufficient buffer for a 1:25 dilution. The tissue was ground in the buffer and inoculated to guar leaves immediately after being cooled. In each of 4 trials a 10 min. exposure to 54° C. inactivated the virus, and in 3 of the trials 52° C. inactivated the virus. While a thermal inactivation point of 54° C. is not unusually low, the use of intact tissue rather than extracts make comparisons with other viruses of doubtful value.

INACTIVATION OF THE VIRUS BY HEALTHY ROSE EXTRACT.—The low infectivity of extracts of infected rose leaves indicated a low virus content or the presence of an inactivator. To test the latter possibility extracts of healthy rose leaves were prepared by grinding a known weight of leaf tissue with a measured volume of buffer. In 4 trials the average number of lesions per half-leaf of guar produced by 1:25 extracts of cowpea leaves containing 10, 5, and 2.5 per cent rose leaf extract were 0.5, 2.4, and 1.0, respectively. The control, with no added rose extract, gave an average of 43 lesions per half-leaf. Evidently part of the difficulty in mechanically transmitting the virus from rose is due to an inactivating effect of the extract.

DISCUSSION.—Rose mosaic virus is similar to a number of other plant viruses in rapidly losing infectivity in plant extracts. Like the viruses of tomato spotted wilt, tobacco streak, and potato yellow dwarf, the presence of a reducing agent prolongs virus activity in extracts. Evidently all these viruses are sensitive to oxidative changes. There are apparently additional factors which tend to inactivate rose mosaic virus, and which have not yet been defined. The apparently permanent loss of infectivity in undiluted extracts is evidence of an unusual sensitivity, as is the fairly rapid loss of infectivity even in the presence of reducing agents. Possibly the sensitivity of the virus to divalent metallic ions may be related to some of the rapid loss of infectivity in extracts. The relation of various salts to infectivity seems worthy of further investigation.

Transmissibility of rose mosaic virus is affected not only by detrimental qualities of the rose extracts, but also by factors peculiar to the virus itself. Considering the combination of a highly labile virus in an extract known to have inactivating properties, it is not surprising that mechanical transmission often is difficult. That it is possible at all seems to be due in part to the greater susceptibility of certain herbaceous hosts, particularly cowpea.

While the herbaceous *Potentilla* spp. were more readily infected than was *Rosa setigera*, no rosaceous species could be infected consistently. On the other hand, transfer of the virus from infected rosaceous plants to guar and cowpea was accomplished fairly readily. The resistance of rosaceous species to infection by mechanical inoculation probably is also a factor tending to lower the chances of securing infection in intra-species transfers with rose mosaic virus.

SUMMARY

Rose mosaic virus was transmitted by mechanical inoculation from *Rosa setigera* to cucumber, where it caused chlorosis, necrosis, and death, and to cowpea, where it caused chlorotic ringspotting and eventual death. From infected cowpea the virus was transmitted to 25 plant species in 7 families, including the original host. It was not transmitted by dodder or by aphids.

Local lesions were produced on guar, which was used as an assay host. Undiluted juice of diseased cowpea leaves was non-infectious, as was juice diluted with buffer after extraction. Cowpea leaves macerated in buffer provided virulent inoculum. Such extracts became inactive within 1 hr. unless reducing agents were present, which extended the longevity to 6 hr. The presence of divalent metallic ions in extracts prevented or greatly reduced infection. In unmacerated cowpea tissue the virus was inactivated by 54° C. in 10 min. Unlike the viruses of tomato spotted wilt and potato yellow dwarf, rose mosaic virus could not be preserved by drying tissue in a partial vacuum at 1° C. with a desiccant, and is apparently less stable than these viruses. Addition of 2.5 to 10 per cent healthy rose leaf extract to infectious extracts of cowpea leaves inactivated most of the virus.

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THE EFFECT OF CERTAIN WATER-SOLUBLE COMPOUNDS ON VIRUS INFECTION [1]

M. Weintraub, J. D. Gilpatrick and R. S. Willison [2]

Considerable attention has been given recently to inactivation of plant viruses and chemotherapy of the diseases they cause. For the most part, *in vitro* techniques have been used, such as mixing of virus and certain compounds previous to inoculation (2, 3, 5, 13, 15). Some workers (7, 8, 9, 10, 12, 14) have attempted to make the host tissue the site of contact between virus and compound.

Many *in vivo* techniques, although suitable for certain purposes, are too laborious for use when large numbers of compounds are to be tested. For this purpose, a relatively simple and rapid technique is required to screen out inactive compounds.

MATERIALS AND METHODS.—Partially purified tobacco mosaic virus was diluted to such a point that usable numbers of local lesions were generally produced on *Nicotiana glutinosa* L. In all cases, carborundum was added to the inoculum. Tobacco mosaic virus was chosen for its stability, which ensured a relatively constant virus titer for a reasonably long period. A limited number of experiments were also carried out with carnation mosaic virus.

The effect of each compound was tested on half-leaves before and after inoculation. For treatment before inoculation, 4 detached leaves of *N. glutinosa* were split along the midribs, and the halves notched for identification. Two left and 2 right half-leaves were floated in a Petri dish containing 25-30 ml. of the test compound, and the corresponding halves in a dish containing distilled water. After 24 hr., the half-leaves were removed, washed thoroughly to prevent possible surface inactivation of the virus, and blotted gently. Each half-leaf was then rubbed with the virus solution, the same number of strokes of the finger being applied to each half-leaf. The half-leaves were then rinsed immediately, and replaced in their respective Petri dishes.

For treatment after inoculation, the half-leaves were inoculated immediately after separation from the plant. They were then rinsed and placed for 6 hr. in distilled water. Two left halves and 2 right halves were then transferred to plates containing the test compound. The corresponding control halves remained in water. In certain cases, the experimental half-leaves were floated on the test compound immediately after inoculation (Table 2).

Half-leaves tended to become water-soaked and short-lived under conditions of variable illumination and temperature. Most of the experiments, therefore, were conducted in air-conditioned chambers, at a constant temperature of 75°F., and illuminated for 10 hr. daily at a constant intensity of approximately 1200 foot-candles. To minimize water-soaking, the plants were placed in a dark, cool corridor for 24 hr. before leaves were detached.

TABLE 1.—*Effect of various compounds on production of lesions by tobacco mosaic virus on detached half-leaves of Nicotiana glutinosa.*

Compound	Concentration	Treatment before inoculation: Mean % reduction	Treatment after inoculation: Mean % reduction
Creatinine	1:100[a]	100	.....
zinc chloride	1:1000	97.0	97.0
	1:10,000	N.S.R.[b]	68.2
	1:100,000	N.S.R.	N.S.R.
Creatinine	1:1700	N.S.R.	.....
	1:17,000	N.S.R.	.....
Zinc chloride	1:2500	79.3	55.8
	1:10,000	53.6	.....
	1:25,000	N.S.R.	.....
Zinc sulphate	1:2500[a]	83.5	60.4

[a] Injury of leaf induced by compound.
[b] N.S.R.—No significant reduction.

RESULTS.—Data summarized in Tables 1, 2, and 3 were analyzed statistically for significance of differences between treated half-leaves and water controls after the transformation suggested by Kleczkowski (6) was applied. In this transformation, $Y = \log (x + c)$, where x is the number of lesions, and c is a constant. The latter c was arbitrarily taken as 1, but essentially the same results were obtained with $c = 5$ or $c = 20$.

EXPERIMENTS WITH TOBACCO MOSAIC VIRUS.—*In vivo.*—Of 54 compounds tested, 50 exhibited no significant effect on the numbers of lesions at 1 or more concentrations. These ineffective compounds were: glycine, dl-alanine, dl-valine, L-(—)-leucine, dl-isoleucine, dl-serine, dl-threonine, dl-phenylalanine, l-tyrosine, dl-methionine, dl-aspartic acid, l-glutamic acid, dl-lysine, dl-cystine, dl-proline, dl-histidine monohydrochloride, dl-tryptophane, asparagine, dl-sarcosine hydrochloride, protamine sulphate, cysteine hydrochloride, dl-ornithine,

TABLE 2.—*Effect of Vitamin B, on production of lesions by tobacco mosaic virus on detached half-leaves of Nicotiana glutinosa.*

Concentration	Treatment before inoculation: Mean % reduction	Treatment 6 hours after inoculation: Mean % reduction	Treatment immediately after inoculation: Mean % reduction
1:1000	91.9	N.S.R.	73.5
1:5000	89.5	.....	.....
1:10,000	22.1	24.5	N.S.R.
1:100,000	N.S.R.	N.S.R.	.....

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TABLE 3.—*Effect of two compounds on production of lesions by carnation mosaic virus on detached half-leaves of Dianthus barbatus (Treatment before inoculation only).*

Compound	Concentration	Mean per cent reduction
Zinc chloride	1:1000	82.5
	1:2500	68.1
Vitamin B_1	1:1000	N.S.R.

betaine, l-taurine, L-(+)-arginine monohydrochloride, riboflavin, nicotinic acid, calcium pantothenate, pyridoxine hydrochloride, i-inositol, para-aminobenzoic acid, folic acid, Vitamin B_{12}, ascorbic acid, ribose nucleic acid and desoxy-ribose nucleic acid (each in 5% NaCl), 3, 4, dl-dihydroxy-phenylalanine, para-aminosalicylic acid, para-nitrobenzeneazoresorcinol, diphenylamine, creatinine, creatine, zinc oxide, zinc phosphate, zinc ethylene bis-dithiocarbamate (65%), n-octadecyl trimethylammoniumpentachlorophenate (40% isopropanol solution), potassium chloride, barium chloride, sodium sulphate, and calcium sulphate.

Three compounds, Vitamin B_1 (thiamine hydrochloride), zinc chloride, and creatinine zinc chloride, were very effective in reducing the number of lesions. As shown in Table 1, 1:2500 zinc chloride and 1:1000 creatinine zinc chloride were effective both before and after inoculation, but the former was less effective after inoculation than before. This difference is difficult to explain since the amount of zinc chloride in a 1:1000 dilution of creatinine zinc chloride is about the same as that in a 1:2500 dilution of zinc chloride, and since creatinine alone appeared to exert no effect on the virus, at least before inoculation. In both cases, inhibitory activity varied almost directly with dilution of the compound. No explanation can as yet be offered, however, for the high reduction (68.2%) produced by creatinine zinc chloride at 1:10,000 when half-leaves were treated after inoculation.

In an effort to determine whether the zinc or the chloride ion was responsible for the inhibitory activity, 4 zinc compounds and 2 chlorides were tested. Five of these, zinc oxide, zinc phosphate, zinc ethylene bis-dithiocarbamate, potassium chloride, and barium chloride were ineffective. The fourth compound, zinc sulphate, was fully as active as zinc chloride (see Table 1). With both compounds, treatment before inoculation appeared to be more inhibitory than treatment after inoculation. However, zinc sulphate induced considerable injury, shortly after treatment was begun.

Neither sodium sulphate nor calcium sulphate, which were tested to determine the efficacy of the sulphate ion, showed any inhibitory activity.

The effectiveness of Vitamin B_1 as an inhibitor of tobacco mosaic virus is shown in Table 2. It is evident that this compound at a concentration of 1:1000 almost completely inhibited production of lesions, both before inoculation and when treatment was begun immediately after inoculation. There was no significant reduction, however, when the half-leaves were inoculated 6 hr. before treatment. At 1:5000, the effectiveness of Vitamin B_1 dropped rapidly. At a dilution of 1:10,000, a small reduction in the number of lesions was observed with treatment 6 hr. after inoculation. This is difficult to explain since the compound showed no significant effects with any other dilutions tested 6 hr. after inoculation.

In vitro.—To determine whether the above compounds were acting directly on the virus, or on the host, several *in vitro* tests were performed. Zinc chloride (1:500) and Vitamin B_1 (1:2500) were mixed with equal portions of the tobacco mosaic virus solution. The treated virus solutions were then applied to half-leaves of *N. glutinosa* at 0, 5, and 24 hr. after mixing. Control halves were inoculated with the untreated virus solution, diluted to the same concentration with water (1:1) There was no significant reduction in the number of lesions produced by the treated virus solution.

EXPERIMENTS WITH CARNATION MOSAIC VIRUS.—Since several compounds had proved effective in reducing infection by tobacco mosaic virus, it was thought advisable to test them against another virus, unpurified virus of carnation mosaic, which produces local lesions on *Dianthus barbatus* (4). Table 3 shows that zinc chloride was equally effective against carnation mosaic virus as against tobacco mosaic virus. Vitamin B_1, on the other hand, caused no significant reduction in the number of lesions, under conditions which had previously been found to inhibit tobacco mosaic virus.

DISCUSSION.—Results described above indicate that the detached half-leaf technique can be used for rapid detection both of inhibitory and of inactive compounds. Of the compounds showing inhibitory activity, Vitamin B_1 seemed the least reliable, since it was ineffective against tobacco mosaic virus 6 hr. after inoculation, and was apparently unable to inhibit carnation mosaic virus at all. However, the effect of this compound in restricting production of lesions by tobacco mosaic virus under certain conditions merits further investigation.

Results with the zinc compounds have a counterpart in the work of Stoddard (14), who found zinc sulphate to be an effective chemotherapeutant against the X-disease of peach. It may be concluded from the data given above that the zinc ion, rather than the chloride ion or sulphate radical, was responsible for the inhibitory action. Consequently, the zinc compound must be in soluble form. In corroboration of this deduction, the 3 relatively insoluble zinc compounds were found to be ineffective in reducing the number of lesions.

Some conclusions may be drawn as to the mechanism of inhibition. Since neither zinc chloride nor Vitamin B_1 inactivated tobacco mosaic virus *in vitro*, it would appear that both exert their effect, not upon the virus directly, but probably through some interaction with the host tissue. Similar conclusions were drawn by Rischkov *et al* (11), when they found that thiamine did not inactivate tobacco mosaic virus *in vitro*, but did produce a decrease in virus content *in vivo*.

From the data in the present paper, it would appear that zinc chloride and zinc sulphate may lag 6 hr. behind the virus in entering the tissues, and still exercise inhibition. Vitamin B_1, on the other hand, inhibits lesion production only with treatment before, or with treatment immediately after inoculation, while a 6-hr. differential in favor of the virus eliminates this activity. Bawden has suggested (1, p. 278) that virus infection may occur in 2 stages, the first being entry into cells and the attachment of virus particles to suitable sites, the second, multiplication leading to lesion production. When it is recalled that inoculation by mechanical rubbing with virus is usually followed by rinsing of inoculated leaves, without hindering infectivity, it seems probable that the first stage hypothesized by Bawden occurs quite rapidly. On this basis, the effectiveness of the zinc compounds both before, and 6 hr. after, inoculation could better be ascribed to their effect on the multiplication stage. If, on the other hand, establishment of the virus requires up to 6 hr., it is possible that the zinc compounds are active in the first stage of virus infection. It may be inferred, by similar reasoning, that Vitamin B_1 may interfere in some way with the first stage, that is, entry and establishment of the virus at sites suitable for multiplication.

SUMMARY

A method for testing the effectiveness of compounds as virus inhibitors, which saved both time and materials, depends upon the inoculation of detached half-leaves, the treated half being floated on the test compound, and the control half on water. Of 54 compounds tested, 3 have been rapidly selected for their ability to reduce significantly the numbers of lesions produced by tobacco mosaic virus on *Nicotiana glutinosa*. Zinc chloride and zinc sulphate are capable of reducing the number of lesions by almost 100 per cent. The zinc ion appears to be the inhibitory agent in these compounds. Zinc chloride also reduces the activity of carnation mosaic virus in *Dianthus barbatus*. Vitamin B_1 is not consistent in its behavior with tobacco mosaic virus and appears to be inactive against carnation mosaic virus.

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ELECTRON MICROSCOPY OF CUCUMBER VIRUS 1[1]

W. H. Sill, Jr., W. C. Burger, M. A. Stahmann, and J. C. Walker

Bawden (1) and Johnson (3), in studies of the common cucumber mosaic virus, reported the presence of rod-shaped particles which occurred rather sparsely in their electron photomicrographs. More recently DeBruyn Ouboter, Beijer and Van Slogteren (2), using a virus believed to be cucumber virus 1, found spherical particles in preparations from tobacco (*Nicotiana tabacum* L.), but there appeared to be some question as to the identity of the virus used in their study. The present paper is a report of studies carried out with a known strain of cucumber virus 1.

EXPERIMENTAL RESULTS.—The virus used in this study was a strain of cucumber virus 1 designated as Y in University of Wisconsin cultures (4). Extracts for infectivity assays and electron microscope mounts were made from tobacco and cucumber (*Cucumis sativus* L.) tissues by grinding in a mortar and pestle. The macerated tissue was filtered through glass wool and the extract was diluted with 0.1 M phosphate buffer, pH 7.0. To maintain optimum infectivity the dilution used for infectivity assays was usually 1 to 10, but occasionally as much as 1 to 100. The diluted extract was centrifuged using gravitational fields which varied from 3,000 × G to 80,000 × G for 80 min. at room temperature in a Servall SS-2 centrifuge or a Grebmeier air-driven centrifuge. Infectivity of aliquots of the various resuspended pellets and supernatant liquids was determined by a local-lesion host assay on cowpea (*Vigna sinensis* Sdvl.), var. Black, according to methods described by Sill and Walker (6). Frozen-dried electron microscope mounts, prepared by methods described by Stahmann, Hagedorn and Burger (7) were made from the same aliquots. In the initial experiments parlodion was used in the preparation of the membranes for the electron microscope mounts, but "formvar" membranes, prepared from 0.2 per cent solution in ethylene dichloride, were used after the latter was found to produce more durable membranes. Dried mounts were shadowed with uranium (20 to 25 A) at an angle of approximately 26° and were examined with an R.C.A. type EMU electron microscope.

Initial preparations were made from extracts of whole infected cucumber plants but assays made from these always gave very low numbers of lesions per leaf owing to the effect of an inhibitor in cucumber previously reported (5). Electron photomicrographs of extracts from the entire infected cucumber plants showed no unique particles when compared with mounts prepared from normal cucumber plant tissue extracts.

Infectivity of the uncentrifuged extracts from diseased *Nicotiana tabacum* L. plants was always high, averaging about 125 lesions per leaf, but upon centrifugation the virus appeared to sediment with the cellular debris, particularly with the chloroplasts and chloroplast fragments. Very little, if any, virus remained in the supernatant liquids when they appeared to be free of chloroplast fragments. The virus was completely sedimented within 80 min. at 5 to 10,000 × G. Electron microscope mounts prepared from the resulting infectious sediment contained some relatively large clumps of what appeared to be small, spherical particles, surrounded and overlaid by amorphous materials. Mounts prepared from non-infectious supernatant liquids, free of any chloroplast debris, contained no comparable aggregates. Similar preparations from normal tobacco plants also contained no such aggregates or other comparable particles. A few rod-shaped particles were seen in both infected and healthy preparations from tobacco but their presence could not be correlated with infectivity.

Preparations from infected cucumber corolla extracts have been reported to contain little or none of the virus inhibitive substance found in the remainder of the plant (5). In contrast to tobacco extracts, where all of the infectivity sedimented within 80 min. at 10,000 × G, the infectivity of the cucumber corolla extracts did not sediment appreciably at 18,000 × G. Even after centrifugation at 36,000 × G for 80 min., the supernatant liquids produced lesion averages of about 30, and occasional lesions were produced from supernatant liquids after centrifugation at 80,000 ×

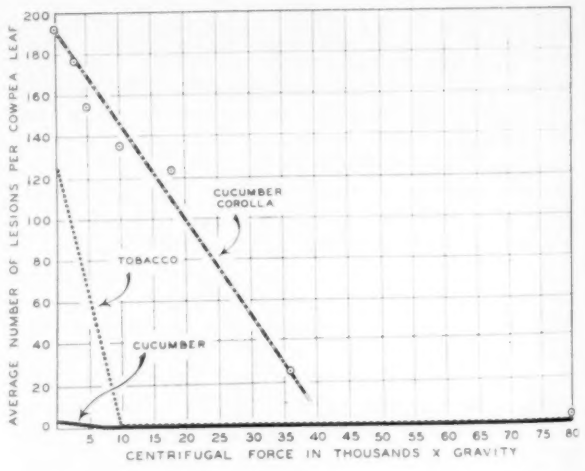

FIG. 1. Comparative Infectivity of Supernatants from Infected Tissue Extracts subjected to various centrifugal forces for 80 min.

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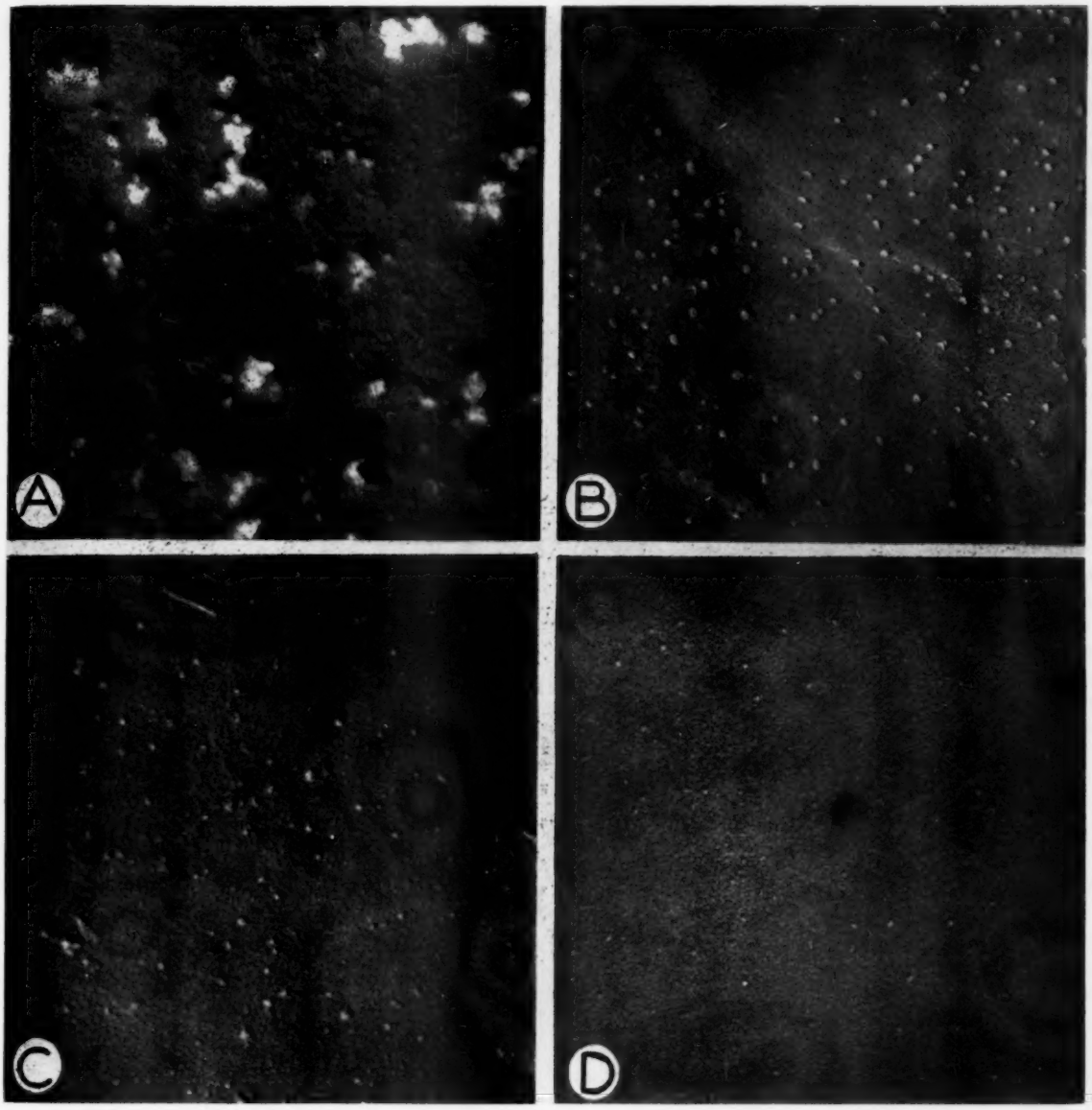

FIG. 2. Electron photomicrographs of supernatants from extracts of cucumber corolla tissue (approximately 23,000 ×). A. Infected extract diluted 1 to 5 and centrifuged at 9,000 × G; aggregations of particles are overlaid and surrounded by amorphous cellular material. B. Infected extract diluted 1 to 50, centrifuged at 9,000 × G, frozen dried on parlodion membrane; note separate spherical particles free from amorphous material. C. Infected extract diluted 1 to 100, centrifuged 9,000 × G and frozen dried on a "formvar" membrane; note rod-like particle and preponderance of spherical particles. D. Healthy extract diluted 1 to 5, centrifuged at 9,000 × G and prepared as in C. The sparse spherical particles, much smaller than those in the infected extract, are characteristic of healthy corolla.

G for the same length of time. This marked difference in the centrifugal forces required to sediment the virus infectivity from cucumber corolla extracts and tobacco and cucumber leaf extracts is shown in Figure 1. The virus activity in corolla extracts sedimented over a wide range of centrifugal forces in marked contrast to the virus activity in tobacco extracts which was completely sedimented by relatively low gravitational forces.

Representative electron photomicrographs which were prepared from infected and normal cucumber corolla tissues are shown in Fig. 2. In photomicrographs made from low speed 3 to 5,000 × G supernatants of the infected cucumber corolla extracts aggregated spherical particles generally predominated. These aggregates were partially obscured by amorphous materials but the aggregates usually were not so large as those seen in preparations from infected tobacco tissue. In higher speed supernatants (9,000 × G) aggregates appeared at times (Fig. 2A) but unaggregated spheri-

cal particles were also seen (Fig. 2B.) Still higher speed supernatants seemed to have less particle aggregation and more of the unaggregated spherical particles. These particles varied somewhat in size and shape but averaged approximately 350 A in diameter. There was a constant correlation between the presence of these particles and high infectivity on cowpea. No such correlation was found with the presence of the occasional rod-shaped particles which were suggestive of those described by Bawden (1) and by Johnson (3). Control mounts prepared from healthy corolla supernatants did not reveal comparable spherical particles (Fig. 2D), but occasional rod-shaped particles, similar to that in Figure 2C, were seen.

DISCUSSION AND SUMMARY

Electron photomicrographs presented seem to show that cucumber virus 1 is a spherical particle having an approximate diameter of 350 A. The majority of preparations from corolla of infected cucumber plants contained aggregates of characteristic ovoid to spherical particles. These particles were not present in mounts prepared from comparable healthy corolla extracts, and no comparable particles were seen in infected cucumber leaf preparations. Infectivity of the cucumber leaf extracts was extremely low owing to the presence of a virus inhibitor in cucumber. In mounts made from infected tobacco leaf preparations spherical particles seemed to be aggregated in large irregular clumps generally overlaid and surrounded with amorphous materials. Most of the infective material in tobacco leaf extracts was sedimented within 80 min. at 5.000 × G and all of it was sedimented at 10.000 × G. Infected cucumber corolla extracts required high speeds (36,000 × G) for sedimentation of most of the virus within 80 min. This behavior suggested that the virus in the tobacco leaf extracts was highly aggregated or was combined with leaf tissue components. A correlation was noted between infectivity and the presence of green tissue fragments in differentially centrifuged leaf extracts. These observations and the fact that the infective fractions from tobacco generally contained much chloroplast material (3,000 × G pellets) suggests that the virus may be combined with chloroplasts or other tissue components, and consequently is sedimented along with those fragments.

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BASIS OF RESISTANCE IN GRAM TO MYCOSPHAERELLA BLIGHT[1]

Abdul Hafiz

In the Punjab, Pakistan, the crop of gram or chickpea (*Cicer arietinum* L), second in area to wheat and of great economic importance, suffers severely from blight caused by the fungus *Mycosphaerella rabiei* Kovaceveski (*Ascochyta rabiei* (Pass.) Labrousse. Among 392 types of gram examined for blight reaction, only 3 have been resistant. They were supplied by the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., under the name Poischishes, as No. 4732, 199 and 281, but have been re-named F8, F9 and F10 respectively. In the field these types flourish under severe epidemics of blight when Punjab types 7 and 15 are killed. Luthra, Sattar and Bedi[2] reported that F8, F9 and F10 are resistant at all stages of growth and under many different environmental conditions.

This paper is based on investigation of morphological and physiological characteristics of 2 resistant types, F8 and F10, and 2 susceptible types, Punjab 7 (P. 7) and Punjab 15 (P. 15), with a view to determining the basis of resistance in types resistant to blight.

A culture of *Mycosphaerella rabiei* was isolated from diseased gram twigs from Lyallpur, Pakistan. Stock cultures of the fungus were grown on oatmeal agar. Spore suspensions for inoculation were prepared in the following manner: surface growth of pycnidia and exuding pycnidiospores was scraped from cultures 15-20 days old, suspended in water, filtered through fine muslin cloth to free it of mycelial

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The author is indebted to Professor W. Brown, F.R.S., Head of Botany Department, Imperial College of Science and Technology, London, for guidance and valuable criticism throughout the course of this work. Thanks are also due to Dr. A. Sattar, Plant Pathologist, Lyallpur, Pakistan, for suggesting the problem.

[2] Luthra, J. C., A. Sattar and K. S. Bedi, Determination of resistance to blight disease (*Mycosphaerella rabiei* Kovaceveski *Ascochyta rabiei* (Pass.)) Labrousse in gram types. Indian Jour. Agri. Sci. 2: 249-264. 1941.

debris while retaining the spores, thoroughly washed 3 times and centrifuged to remove traces of nutrient. A water suspension of spores was prepared to contain approximately 20-30 spores per microscopic field under high power. Gram plants were atomized with the freshly prepared suspension, then covered with bell jars for 72 hr. in order to maintain high percentage of humidity.

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS.—No appreciable differences in height, or numbers of branches, leaves and leaflets, of resistant and susceptible types of gram were found except that the number of tertiary branches is from 1.8 to 2.2 times greater in susceptible types. This larger number of tender tertiary branches may possibly explain the pronounced symptoms of the disease on susceptible types in nature, even in early stages.

The hair population on stems and leaves of resistant types was observed to be greater than that of susceptible types; from 1.8 to 2.0 times greater for stems and 1.3 to 1.5 times greater for leaves. Since the glandular hairs are known to secrete malic acid[3] which in turn is known to affect spore germination, this differential in hair population may in part account for the difference in susceptibility of resistant and susceptible types of gram.

Cuticle thickness varied only slightly in the gram types under study, being 0.010 mm. in P7, 0.016 mm. in F8 and 0.011 mm. in F10. Slightly greater thickening in resistant types may be responsible for slow penetration of fungal hyphae, but no detailed studies of penetration were made.

The number of stomata varied from 66 to 96 in P7, and from 114 to 136 in resistant types. The higher number of stomata in leaves of resistant grams does not appear to favor invasion by the fungus, as fungal penetration takes place through the epidermis rather than through the stomata.

The percentage of dry matter and the moisture content, being the same for all types of gram, apparently do not affect susceptibility to the fungus.

There was little difference in acidity of cell saps of P7 (pH 6.09), F8 (pH 6.84) and F10 (pH 6.64) and it was found that these slight differences between resistant and susceptible grams did not affect germination of the fungus spores or growth of mycelium. Nor did total nitrogen content, equal in all the grams, influence growth of the fungus.

SPORE GERMINATION.—Germination of the fungus spores was tested in distilled water, in different strengths of malic acid, in cell sap solutions from different gram types, in gram seed extracts, and in drops of distilled water sprayed on the foliage of gram plants at various stages of growth and collected after 24 hr. Three uniform drops of a germination medium were placed on a glass slide, the spores were added, and the slides were kept at 100 per cent relative humidity at 20°C.

High percentages of spores germinated in all cell sap solutions and in gram seed extracts, no differences being detected in relation to the type of gram used. Results of tests in other media are in Tables 1 and 2. Spores germinated more rapidly in dilute malic acid solutions at pH 4.44 or 3.44 than in distilled water at pH 7 but very few spores germinated in 2 and 3 per cent malic acid solutions (pH 2.27 and pH 2.12). Germ tubes rarely developed beyond a budding stage. Adding glucose to the stronger malic acid solutions did not improve spore germination.

Spores also germinated well in water drops removed from the foliage of young gram plants of all the varieties. They germinated fairly well, sometimes more slowly, in water drops removed from foliage of older plants of the susceptible gram type P7. Production of malic acid increases with age of gram plants[3], a fact that may account for poorer germination and slower growth of germ tubes as the resistant gram F10 ages.

[3] Sattar, A.—On the occurrence, perpetuation and control of gram (*Cicer arietinum* L) blight caused by *Ascochyta rabiei* (Pass.) Labrousse, with special reference to Indian conditions. Ann. Appl. Biol. 20: 612-32, 1933.

TABLE 1.—*Germination of spores of Mycosphaerella rabiei in various media*

Medium	Percentage germination[a] 24 hr.	80 hr.	Germ tube development 24 hr.	80 hr.
Distilled water, pH 7	65	100	4.2 μ	Long, branched
Malic acid				
0.001 per cent, pH 4.44	97	100	18.6 μ	Long, branched
0.01 per cent, pH 3.44	80	100	4.2 μ	Long, branched
0.05 per cent, pH 3.01	45	96	4.2 μ	18.5 μ
1.00 per cent, pH 2.38	42	53	Buds	13.3 μ
2.00 per cent, pH 2.27	Tr.	Tr.	----	----
3.00 per cent, pH 2.12	0	Tr.	----	buds
Glucose, 2 per cent	73	----	22.1 μ	----
Glucose plus malic acid:				
2 per cent: 1 per cent	51	64	2.7 μ	2 to 8 μ
2 per cent: 2 per cent	Tr.	Tr.	----	----
2 per cent: 3 per cent	0	Tr.	----	buds

[a] Counts based on 100-150 spores.

TABLE 2.—*Germination of spores of Mycosphaerella rabiei in media that had been on host foliage for 24 hr.*

Germination medium[a]		Percentage germination[b] 24 hr.	Germ tube development 24 hr.
Spore suspension, after 24 hr. on:			
F10 foliage	25 days old	80	26 μ
	46 days old	22	buds
	88 days old	Tr.	----
P7 foliage	25 days old	88	35 μ
	46 days old	98	33 μ
	88 days old	43	15 μ
Water drops, after 24 hr. on:			
F10 foliage	10 days old	94	64 to 128 μ
	42 days old	51	9 to 52 μ
	57 days old	42	buds
	78 days old	0	----
P7 foliage	10 days old	99	64 to 192 μ
	42 days old	62	22 to 132 μ
	57 days old	76	35 μ
	78 days old	39	7 to 26 μ
Distilled water control		64	4 to 8 μ

[a] The spore suspension and the water were sprayed on foliage of plants kept at an absolute relative humidity for 24 hr., then uniform drops taken from the foliage were put on glass slides and the spores germinated therein.
[b] Counts based on 100-150 spores.

DEVELOPMENT OF THE DISEASE.—Gram plants at various stages of growth were inoculated by spraying their foliage with a fungus spore suspension. Plants of both the resistant and susceptible gram types, if less than 40 days old, were attacked with equal intensity (100 per cent). On 78-day-old plants of the resistant gram types the intensity of attack was 50–57 per cent as compared to 100 per cent on plants of the susceptible gram types. Another difference between older plants of the gram types lay in the rapidity with which host tissue was killed. Infected tissue in susceptible types was killed within 9–14 days, whereas about half the infected tissue in the resistant types was not killed and the rest was dead only after 15–30 days. In an experiment with 40-day-old plants an attempt was made to determine the minimum time required by the fungus for penetration of susceptible and resistant types of gram. Plants were sprayed with spore suspension and then, after intervals ranging between 24 and 96 hr., were thoroughly washed with water in order to remove the inoculum. The infection percentages on plants in these series indicate that the fungus penetrates and infects a susceptible host much more rapidly than a resistant host (Table 3).

TABLE 3.—*Percentages of infection on 3 gram types, with inoculum that remained on foliage of the 40-day-old plants for various times*

Gram host	Percentage of infection caused by inoculum on foliage for				
	24 hr.	36 hr.	48 hr.	72 hr.	96 hr.
P7 (susceptible)	100	100	100	100	100
F8 (resistant)	0	14	45	85	100
F10 (resistant)	0	7	33	71	100

DISCUSSION.—In nature, *Mycosphaerella* blight usually appears in fields when plants are of advanced age, because rainfall is abundant then and pycnidia burst to liberate spores which find temperatures suitable for germination. But in spite of the favorable external environment, the presence of numerous glandular hairs and consequent increased malic acid secretion on resistant gram types inhibit spore germination and retard growth of the fungus.

The fungus penetrates and infects 100 per cent of the susceptible grams if inoculum remains on the plant foliage for 24 hr., but that time is insufficient for infection of the resistant grams. Such a differential infection occurs only on aging plants, for grams of all types less than 40 days old are equally liable to infection. In nature the period of juvenile susceptibility does not coincide with the period of favorable environment for infection.

Thus a resistant gram type in its juvenile stage may escape infection by *Mycosphaerella* because of lack of inoculum and because low temperature and low humidity do not favor infection. Its resistance in later growth stages may be attributed to the large number of glandular hairs on stem and leaf surface and their secretion of malic acid in concentrations detrimental to fungus spore germination and germ tube growth; to the long time required by the fungus for penetration and infection of the host; and to the slow development of the fungus and the disease symptoms within the host tissues.

SUMMARY

Studies carried out on morphological and physiological characteristics in gram types susceptible and resistant to *Mycosphaerella* blight have shown that hair population, being higher in resistant types, is responsible for greater secretions of malic acid in later growth stages and that this high concentration of malic acid is detrimental to fungus spore germination and germ tube growth. Moreover, it has also been noticed that comparatively longer time is needed by the fungus to penetrate the resistant types and produce disease symptoms. However, in early stages of plant growth there is no difference in the susceptibility of resistant types and susceptible types because of low malic acid production. Thus, in nature, resistant types escape infection in early stages of growth because of lack of inoculum and unfavorable temperature and humidity; but in later stages they become resistant on account of production of malic acid concentrations detrimental to fungus growth, and because of slow penetration of the fungus.

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LOW TEMPERATURE AS A FACTOR IN THE SUSCEPTIBILITY OF MATURE-GREEN TOMATOES TO ALTERNARIA ROT [1]

L. P. McColloch [2] and John T. Worthington [2]

It has long been recognized that susceptibility of tomatoes to decay is increased if held for a week or more at low, but not freezing, temperatures. One of the most prevalent decays under such conditions is alternaria rot caused by *Alternaria tenuis* auct.[4]

The causal fungus is generally considered to be a weak pathogen that ordinarily is unable to produce an active rot on healthy mature-green tomatoes. In the field alternaria rot follows such trouble as sunscald, blossom-end rot, faulty blossom scars, and growth cracks. Thomas[5] reported *A. tenuis* as a cause of "freckle," a superficial spotting or blemish of ripe tomato fruits in the field, but not as a cause of rot. Douglas[3] in 1922 described an alternaria spot which appeared on tomatoes late in the season during wet weather in California. The disease was characterized by brown, circular spots, which became depressed and covered with a velvety mass of the causal fungus. Judging from the illustrations the causal fungus was undoubtedly *A. tenuis*.

Occasional rail shipments have been observed to have a high percentage of tomatoes affected with alternaria rot, especially around the stem scar (Fig. 1). These and other observations prompted studies on the influence of temperature and duration of storage at low temperature on susceptibility of mature-green tomatoes to alternaria rot.

MATERIALS AND METHODS.—A strain of *A. tenuis* isolated from a typical alternaria rot lesion on a tomato grown in Florida was used in the tests herein reported. Similar results were obtained in other tests with all the strains of *A. tenuis* recently reported by

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[2] Respectively, associate pathologist and junior horticulturist, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland.

[3] Douglas, B. A new alternaria spot of tomatoes in California. Phytopath. 12: 146-148. 1922.

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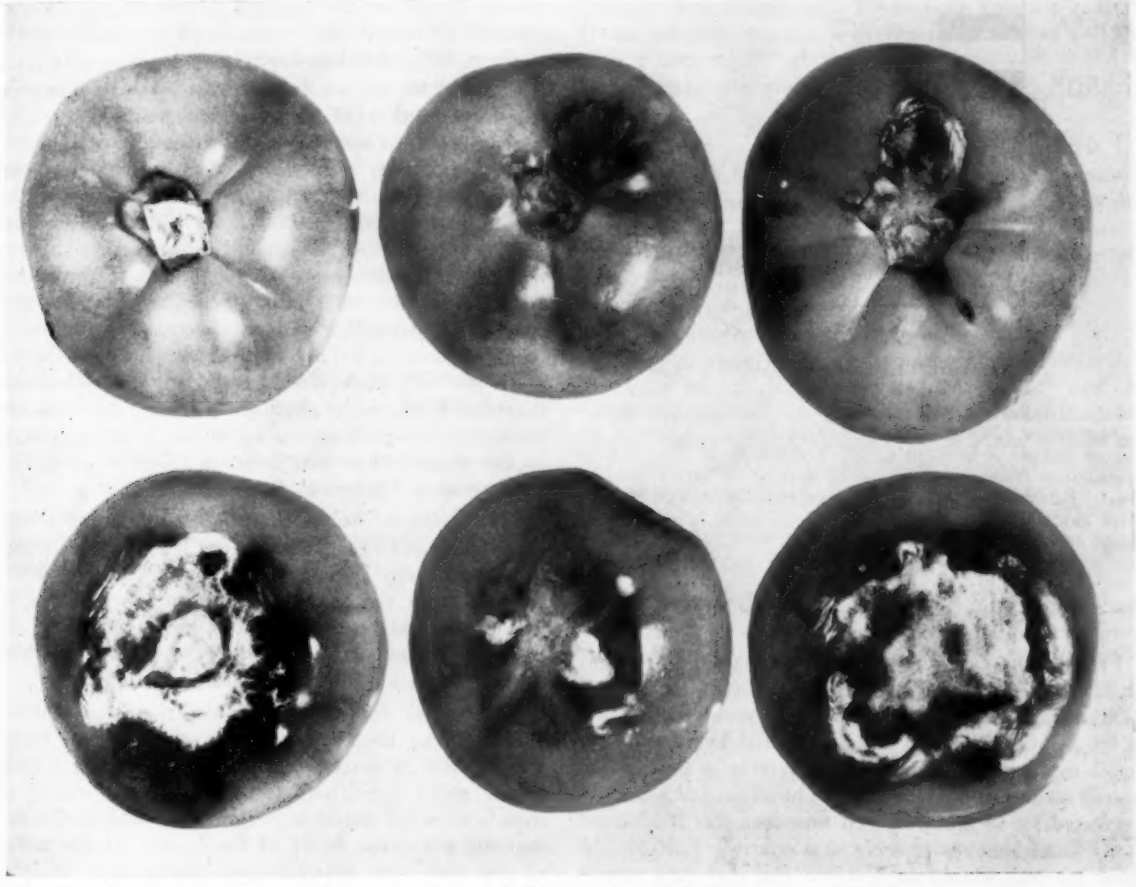

FIG. 1. Alternaria rot on tomatoes in a commercial shipment in which 75 per cent of the fruits were affected.

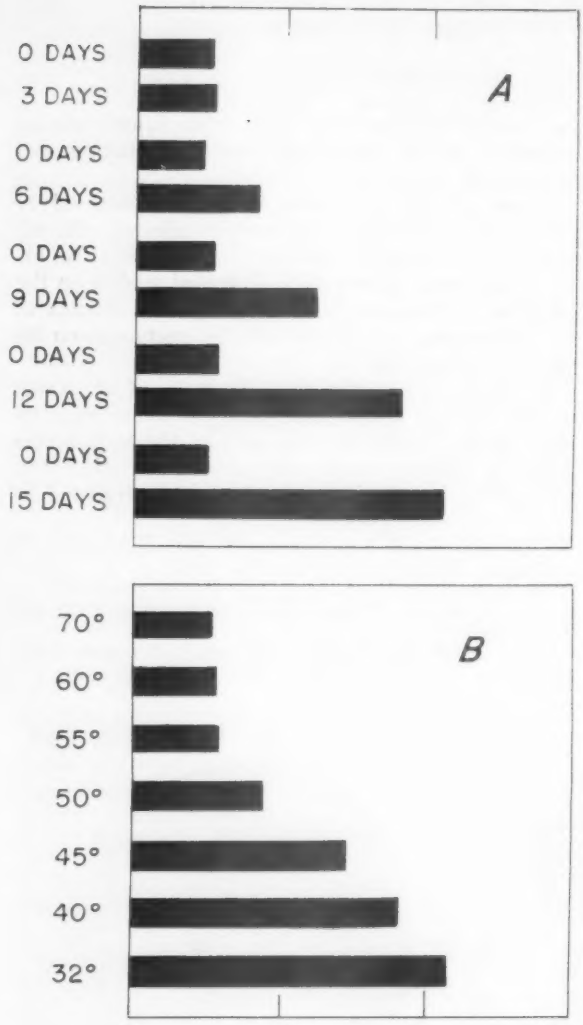

FIG. 2. Influence of temperature on the susceptibility of mature-green tomatoes to *Alternaria tenuis*. A, effect on size of lesions of holding at 32° F. for 0 to 15 days before inoculation (tomatoes held for 14 days at 60° after inoculation). B, effect of various temperatures for initial 14 days after inoculation on size of lesions (tomatoes held an additional 14 days at 60°).

the senior author[4]. Locally grown Rutgers tomatoes were used in all inoculation tests.

Freshly harvested mature-green tomatoes were held at 32° F. for 3, 6, 9, 12, and 15 days. At the end of each storage period the test lot was removed, washed in 95 per cent alcohol, and inoculated by inserting a small mat of mycelium of *A. tenuis* in a pocket-like wound on the side of the fruit. The same day a freshly harvested lot of mature-green tomatoes was inoculated in the same manner to serve as a control. Each inoculated lot was placed in a moist chamber and stored at 60° for 14 days for development of the fungus. At the end of the test the diameters of the lesions were measured. In Figure 2, A, are the average measurements for 29 lesions per treatment, representing 3 separate experiments.

In another group of tests freshly harvested mature-green tomatoes were prepared and inoculated by the method just described. After inoculation each lot was placed in a moist chamber and stored at 32°, 40°, 50°, 55°, 60°, or 70° F. for 14 days and then removed to 60° for an additional 14-day period for the fungus to develop. At the end of the test the diameters of the lesions were measured. In Figure 2, B, are given the average measurements for 32 lesions per treatment, representing 3 separate experiments.

RESULTS.—Results of the 2 types of tests are shown graphically in Figure 2. Figure 2, A, shows the diameters of the lesions that developed when mature-green tomatoes were held at 32° F. for various periods of time and then inoculated and held at 60° for 14 days as compared with freshly harvested tomatoes of the same stage of maturity that were inoculated and held at 60° for 14 days. The data indicate that a slight weakening of the fruits by the cold occurred after 6 days. The degree of weakening is indicated by the diameter of the alternaria rot lesions. The fruits became progressively weaker and the diameter of the lesions larger as the holding period at 32° was increased. None of the controls (fruits held continuously at 60°) developed active rot.

Figure 2, B, shows the influence of temperature on the susceptibility of mature-green tomatoes to alternaria rot. Here are shown the diameters of the alternaria rot lesions that developed when mature-green tomatoes were inoculated and held at a series of temperatures from 32° to 70° F. for 14 days and then held at 60° for an additional 14-day period. There was no active decay on the inoculated tomatoes held initially at 55°, 60°, and 70°. The fungus caused only a dry, darkened blemish often no larger than the inoculation wound (Fig. 3). In the lot held at 50°, there was very slight development of rot which showed that the fruits were slightly weakened by this temperature. Progressive susceptibility to alternaria rot as the temperature was reduced from 50° to 32° is illustrated in Figures 2, B, and 3.

DISCUSSION.—The present studies substantiate previous reports as to the weakness of *A. tenuis* as a tomato fruit pathogen. Some variation may occur in the parasitism in the many strains, but these tests and the unreported ones with other strains indicate that in general they are unable to cause active rot when inoculated into healthy, mature-green tomatoes.

The fungus apparently becomes readily established on the corky tissue around the stem scar of tomato fruits while in the field. Harvested fruits held under warm, moist conditions frequently show around the stem scar scant aerial mycelium and chains of spores that did not cause decay of the fruits. At the margin of the stem scar many tomato fruits show incipient infections that usually remain quiescent unless the

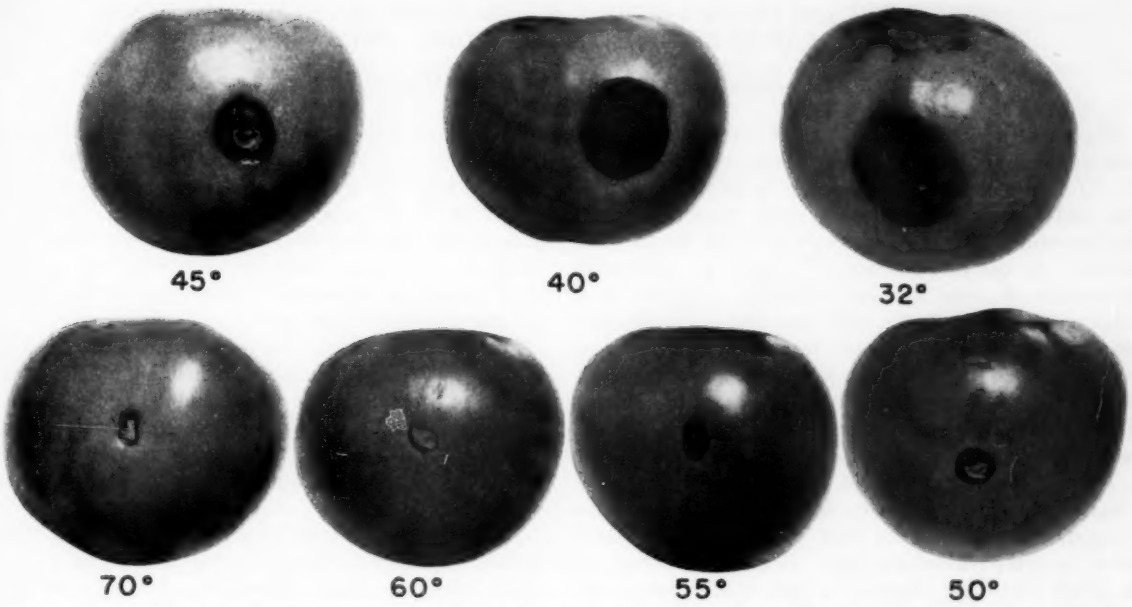

FIG. 3. Influence of temperature on susceptibility of mature-green tomatoes to alternaria rot. Fruits were inoculated and held at indicated temperatures for 14 days and then at 60° F. for 14 days.

fruits are subjected to adverse and weakening conditions such as low temperature.

The following hypotheses have been advanced to account for the extensive decay of mature-green tomatoes that have been held at low temperatures. (1) Resistance of fruit is lowered, (2) infectiveness of fungus is enhanced, and (3) the delay in ripening permits incipient infections to become established.

In order to determine whether fruits were weakened by low temperatures or *A. tenuis* was favored by permitting it to establish incipient infections while the fruits were at low temperatures, the 2 types of tests described were used. In the test where tomatoes were held at 32° F. for different lengths of time and then inoculated after removal to 60°, the fungus was not exposed to low temperature, whereas in the other type of test the tomatoes were inoculated and then held at temperatures of 32° to 70°, thus exposing the fungus inoculum to the same temperatures as the fruits.

The same results were obtained in both types of tests. Tomatoes that were not chilled failed to become actively infected by *Alternaria*, whereas those that were chilled developed alternaria rot in proportion to the length of exposure to 32° F. or to the decrease in temperature below 50°. These tests indicate that the tomatoes were weakened by low temperatures and that the pathogenicity of the fungus was unchanged by such temperatures.

SUMMARY

Alternaria tenuis is recognized as a weak pathogen that is ordinarily unable to produce decay in healthy mature-green tomatoes. The occurrence of severe alternaria rot in certain commercial shipments of tomatoes indicated that low temperature was possibly a factor in susceptibility.

Tests with mature-green fruit held at 32° F. for various periods before inoculation and those with fruit held at various temperatures between 32° and 70° after inoculation indicated clearly that sound, mature-green tomatoes are weakened by holding at low temperatures and that the low-temperature injury causes them to become susceptible to alternaria rot.

PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

SWEET POTATO CLONES WHOSE ROOTS SERVE AS SYMPTOMLESS CARRIERS OF INTERNAL CORK VIRUS [1]

Robert Aycock and Morris B. Hughes [2]

The exact symptomatology of the internal cork disease of sweet potatoes is not well understood. Nusbaum (5, 6) reported that veinclearing, mottling, ringspotting and bronzing of the leaves were associated with the necrotic spotting found in roots of the Porto Rico variety. In contrast Rankin (7) found no consistent association of above and below-ground symptoms in a large number of varieties, selections and seedlings, and Martin (1) suggested that the leaf and root symptoms may be caused by more than one virus, since he found that certain lots of ringspotted plants of the Porto Rico variety did not produce corky roots. These opposing viewpoints indicate the need for further study.

The discovery that certain clones show pronounced ringspotting of leaves but no detectable root symptoms suggested the possibility that roots of some plants serve as symptomless carriers. Grafting experiments in which 2 of these clones (3-6 and 32-149) were used with plants of the susceptible Porto Rico variety are described here.

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Approved by the Director as Technical Contribution No. 194 of the South Carolina Agricultural Experiment Station.

[2] Associate Plant Pathologist and Horticulturist, respectively. The writers are indebted to W. B. Rogers and J. C. Etheredge whose cooperation and assistance made this study possible. The assistance of J. J. Wolfe in preparation of the illustrations is also acknowledged.

MATERIALS AND METHODS.—Clone 32-149 originated from a cross[3] of a seedling of Yellow Yam × Nancy Hall. Clone 3-6 was obtained from a cross of clone 32-149 with an unidentified male parent. Plants of both clones, grown annually for 4 years in close proximity to Porto Rico plants infected with internal cork virus developed abundant foliage symptoms (Fig. 1. A) but no corky lesions.

Lot A, Porto Rico, obtained from Manteca, California, was judged cork-free since ½ of the single bushel of roots available was examined and found free of symptoms. Lot B, Porto Rico, was obtained from Oak Grove, Louisiana. No corky lesions were observed in 515 roots examined during the storage season of 1949-50.

Roots of corky Porto Rico have been grown at the Edisto Experiment Station for the last 4 years. Scattered foliage symptoms have been observed and about 35 per cent of the roots examined prior to this experiment showed corky lesions.

Core-grafting into transversely-halved roots was performed in the usual manner (6) except that all introduced plugs from corky roots contained portions of a corky lesion.

[3] Roots were supplied by Dr. Julian Miller of the Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana, who made the original cross.

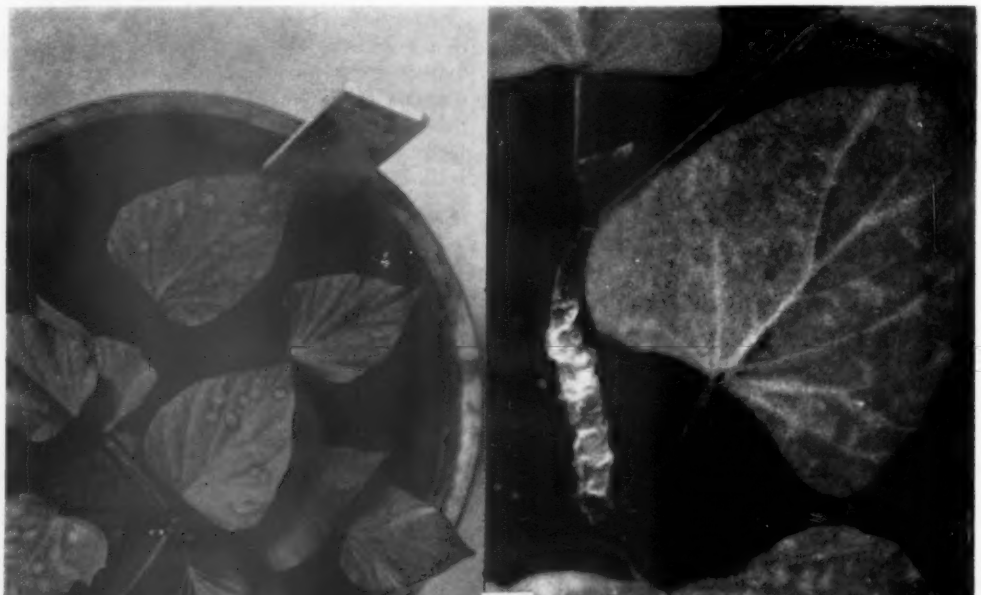

FIG. 1. Ringspotting and veinclearing in sweet potato leaves. A. Ringspotting in leaves of Clone 3-6. The lower leaf exhibits an advanced stage observed just prior to defoliation. B. Veinclearing in an originally cork-free Porto Rico stock inoculated with the internal cork virus by cleft grafting. Note that the center leaf which shows symptoms has arisen from below the site of the graft.

TABLE 1.—*Incidence and severity of internal cork in sweet potato roots harvested from core-graft and cleft-graft-inoculated sprouts and cuttings*

Source of roots	No. of lots sampled [a]	Incidence and severity of internal cork: Per cent lots containing corky roots	Total roots	Per cent roots corky	Average rating of corky slices [b]
Core-grafting experiment					
Series 1:					
Lot A, Porto Rico half-roots inoculated with corky plugs of Porto Rico	19	37	39	31	2.39
Lot A, Porto Rico half-roots	20	0	42	0	----
Series 2:					
32-149 half-roots inoculated with corky plugs of Porto Rico	14	0	57	0	----
32-149 half-roots	11	0	36	0	----
Series 3:					
Lot A, Porto Rico half-roots inoculated with corky plugs of Porto Rico	18	39	31	32	2.38
Lot A, Porto Rico half-roots inoculated with plugs of 32-149	21	19	34	12	2.42
Cleft-grafting experiment					
Lot B, Porto Rico cuttings	15	0	135	0	----
Cuttings from corky Porto Rico	13	77	82	43	2.22
Cuttings from Lot B, Porto Rico scions cleft-grafted to corky Porto Rico stocks	10	90	146	45	2.21
Cuttings from Lot B, Porto Rico scions cleft-grafted to 3-6	7	100	42	41	2.18
3-6 cuttings	12	0	77	0	----

[a] Lots refer to original sprouts from which roots were harvested directly or from which cuttings were made. In both experiments some sprouts failed to survive, others produced no enlarged roots.

[b] According to Nusbaum's (6) scale where 1 = spots less than 1/16 in. in diameter, 2 = spots or aggregates 1/16—¼ in., 3 = spots or aggregates ¼—1 in., and 4 = spots or aggregates greater than 1 in.

All half-roots were bedded in deep flats. When sprouts of sufficient size had developed, 1 from each half-root was set in greenhouse benches. Roots from the various graft combinations were stored at 26-30°C for 3 months to promote symptom expression (2). Roots were then cut transversely into slices ⅛ - ¼ in. thick and examined for lesions. Each slice was rated according to Nusbaum's (6) scale.

Possibility of vector transmission was minimized by growing all material in a greenhouse in which open vents were covered with unbleached muslin. Exhaust fans partly compensated for loss of air circulation. As extra precaution, plants were dusted weekly with 3 per cent lindane and periodically fumigated with Nico-fume and Benzo-fume.

EXPERIMENTAL RESULTS.—Three series of inoculations, each involving 25 roots halved transversely were made to transmit internal cork by core-grafting. In Series 1, corky plugs from Porto Rico roots were introduced into Lot A, Porto Rico half-roots. In Series 2, corky plugs were inserted into 32-149 half-roots. The noninoculated half of each cork-free Porto Rico and 32-149 root served as a control. In Series 3, Lot A, Porto Rico half-roots were core-grafted with 32-149 plugs. The corresponding halves were inoculated with corky plugs from Porto Rico.

Veinclearing, indistinct mottling, and purple ring-spotting were present in about 40 per cent of the Porto Rico plants core-grafted with corky plugs (Series 1 and 3). None of the corresponding controls showed foliage symptoms. About 30 per cent of the 32-149 plants showed foliage symptoms, but plants with such symptoms were approximately as numerous among the control as among the core-grafted lot. Of the Porto Rico plants arising from roots core-grafted with 32-149, 11 per cent also showed foliage symptoms. Incidence and severity of internal cork in roots harvested from the core-grafted lots are shown in Table 1. From the data it is apparent 1) that internal cork was transmitted to a high percentage of Porto Rico plants by core grafting, 2) that 32-149 roots served as symptomless carriers of internal cork virus, and 3) that presence of virus in roots serving as symptomless carriers was detected by core grafting to half-roots of susceptible Porto Rico.

In a parallel experiment to transmit internal cork by means of cleft grafts 16 sprouts each of 3-6, Lot B, Porto Rico and corky Porto Rico were set in large pots. Sprouts from each lot were numbered 1-16, those of corresponding number being used in grafting relations with each other. In the 2 series of grafts, 3-6 and corky Porto Rico sprouts were used as stocks. Two cuttings from each of Lot B, Porto Rico sprouts were obtained. One of these was inserted in a 3-6 stock, the other in a corky Porto Rico stock. After the scions had made sufficient growth, portions of them were rooted, increased by further cuttings, set in soil beds, and allowed to grow to maturity. The cut-

tings removed from 3-6 and corky Porto Rico sprouts prior to their use as stocks were also rooted, increased by further cuttings, set in soil beds, and allowed to produce storage roots. Cork-free controls were obtained in like manner from the original cork-free sprouts.

All corky Porto Rico and all 3-6 sprouts showed foliage symptoms in the early part of the growing period. Although no Lot B, Porto Rico control sprout showed foliage symptoms, about 30 per cent of Lot B scions grafted on either corky Porto Rico or 3-6 sprouts developed symptoms of the type shown in Figure 1, B. Choice of stock apparently did not affect the type of symptoms exhibited by the scion. Incidence and severity of internal cork in the various cleft-grafted lots are shown in Table 1. The data indicate that an even higher percentage transmission of internal cork was obtained by cleft grafting than by core grafting: the apparent superiority of the cleft-graft suggests its use in further research on the internal cork disease. The data also provide evidence that 3-6 roots serve as symptomless carriers of internal cork virus.

Discussion.—The fact that all of the originally cork-free Porto Rico sprouts grafted to 3-6 produced corky roots is strong evidence that the 3-6 clone carries internal cork virus, although symptoms do not develop in the roots.[4]

The production of 1 corky root each by 4 sprouts of cork-free Porto Rico core-grafted with 32-149 is somewhat weaker evidence for the view that 32-149 is also a carrier of internal cork virus. Although the presence of symptoms in these roots could be considered the result of accidental transmission, it would be necessary to assume that 4 accidental transmissions had occurred, since each sprout arose from a separate core-grafted half-root. Absence of symptoms in roots of the Porto Rico control renders this explanation unlikely. Furthermore, absence of lesions in the core-graft inoculated and control lots of 32-149 indicates that internal cork lesions do not develop in the roots of this clone.

Although internal cork virus was recovered from plants which showed brilliant ringspotting but no detectable root lesions, the possible relationship of above and below-ground symptoms remains undetermined. All Lot B, Porto Rico scions which showed foliar symptoms after being grafted to corky Porto Rico or 3-6 stocks, developed corky roots, while the control lots developed neither type of symptom. Corky roots were produced, however, by scions which showed no leaf symptoms after being grafted to 3-6 or corky stocks. Moreover, all sprouts originating from corky slices showed vein-clearing, mottling and ringspotting, but only 77 per cent of them yielded corky roots (Table 1).

Since the environmental factors which affect both types of symptom expression are only partly understood (2, 6) the presence of 1 type of symptom in the absence of the other does not necessarily indicate that more than 1 virus is involved. The possibility is recognized, however, that a symptomless carrier of internal cork virus could also be infected with a second virus causing leaf symptoms.

[4] After this paper was submitted for publication, Nielsen (3) presented evidence which indicates that there are other sweet potato clones whose roots serve as symptomless carriers of internal cork virus.

Summary

No symptoms of internal cork were observed in roots of 2 sweet potato clones (3-6 and 32-149) which exhibit pronounced ringspotting of leaves.

The internal cork virus was, however, transmitted from these clones to cork-free Porto Rico plants by means of core-grafts and cleft-grafts. This indicated that internal cork symptoms do not develop in the roots of these clones, although the causal virus is present. The close relationship of the clones suggests that this characteristic is heritable.

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THE USE OF RADIOCARBON FOR TAGGING FUNGI [1]

H. E. Wheeler

While the value of radioisotopes as biological research tools has been amply demonstrated, their application in the fields of mycology and plant pathology has been limited. Many of the possible applications in these fields seem to be dependent upon the development of methods by which fungi or their metabolic products can be rendered radioactive (tagged). Certain such applications have been suggested; the use of tagged spores for determining spore distribution patterns (1), and the use of tagged mycelium to study sexual interaction (6). Warren (5) has utilized tagged cultures of *Bacterium stewartii* Ewr.Smith to study the distribution of this pathogen in the host. Other possible applications include the use of tagged fungi to investigate problems in host-parasite relationships, particularly those involving the role of extra-cellular enzymes or toxins produced by plant pathogens during penetration or early stages of infection.

The present investigation has been directed toward the development of methods for tagging fungal spores and mycelium with radiocarbon, the determination of the fate and distribution of introduced radioactive material, and studies of its effects upon the growth, pathogenicity, and genetics of the tagged organisms. Abstracts of some of the data to be presented have been published (6, 7).

MATERIALS AND METHODS.—Radiocarbon, in the form of C^{14}-labeled sucrose, was selected chiefly because of the abundant natural occurrence of carbon in fungi and in the products of fungal metabolism. C^{14}-labeled sucrose having specific activities of 0.152, 0.440, and 1.623 microcuries/mg. was incorporated into Fries no. 3 basal medium (3) in such quantities that media having activities of 2.5, 5, 10, 20, and 30 microcuries/ml. were obtained. The sucrose concentration of all media containing radiocarbon was adjusted to 2 per cent by the addition, when necessary, of nonradioactive sucrose. In some cases the basal medium was supplemented with 0.1 per cent yeast extract and 0.1 per cent peptone to stimulate sporulation. The limited availability and high cost of C^{14}-labeled sucrose necessitated the use of fungi that would undergo the desired growth processes on small drops of radioactive media. After screening a large number of fungi, *Glomerella cingulata*, *Botrytis cinerea*, *Helminthosporium victoriae*, *Colletotrichum falcatum*, and a sterile form known as the "blackpatch" fungus[2] (4) were selected.

Three methods for tagging fungi were developed. In the first, a drop of radioactive medium was placed near the center of a glass microscope slide and strips of nonradioactive medium were spread on both sides of the drop, separated from it and from each other by areas of bare glass 2-3 mm. wide. The fungus was then planted on the radioactive drops and allowed to grow across the areas of bare glass onto the strips of nonradioactive medium. In the second method, the fungus was planted in a drop of liquid radioactive medium and allowed to produce a small mat of mycelium. This mat was then removed, placed on filter paper in a Büchner funnel under slight vacuum, and washed, by directing a stream of sterile water onto the mat, until no radioactivity could be detected in the wash water. The third method consisted of planting the fungus on a drop of solid radioactive medium, allowing it to sporulate, harvesting the spores and washing them by repeated centrifugation and resuspension until no radioactivity could be detected in the supernatant. The mycelial mats and spores tagged by the last 2 methods were transferred, after being washed, to a nonradioactive medium (potato-dextrose, oatmeal, or water agar) which had been spread in a thin film on a sterile microscope slide. These slide cultures were allowed to develop in moist chambers for periods ranging from a few min. to 15 days, after which the cultures were killed by heat or by osmic acid fumes and placed for 24 hr. in a drying oven at 80°C. Autoradiographs were then made by placing the dried cultures in contact with photographic emulsions. Control preparations, consisting of cultures grown exclusively on nonradioactive media, were apposed to photographic emulsions for periods equal to those used for making autoradiographs. Eastman Kodak No-Screen X-ray and Nuclear Track (NTB_2) emulsions were used for most of the autoradiographs and the autoradiographic methods employed were essentially those described by Fitzgerald, et al (2).

AUTORADIOGRAPHIC RESULTS.—The autoradiographs (Fig. 1) showed that fungi were tagged effectively by each of the 3 methods. Fig. 1, A-D, are autoradiographs of cultures tagged by planting the fungus on a radioactive drop and allowing it to grow across areas of bare glass onto strips of nonradioactive medium. In cultures tagged by this method, transfer of radioactivity from the drop to the mycelium growing on the nonradioactive medium continues to occur until the culture is killed, thus permitting more extensive development and shorter autoradiographic exposures than are possible with cultures tagged by the other methods. However, this method involves certain difficulties not encountered with the others. One such difficulty is the diffusion of radioactivity from the drop across the bare glass barriers into the nonradioactive medium as a result of condensation of moisture on the slide. While this can be prevented by maintaining the humidity in the culture chamber at a low level, most fungi grow poorly under such conditions and may not cross the bare glass barriers (notice in Fig. 1, B that the strips

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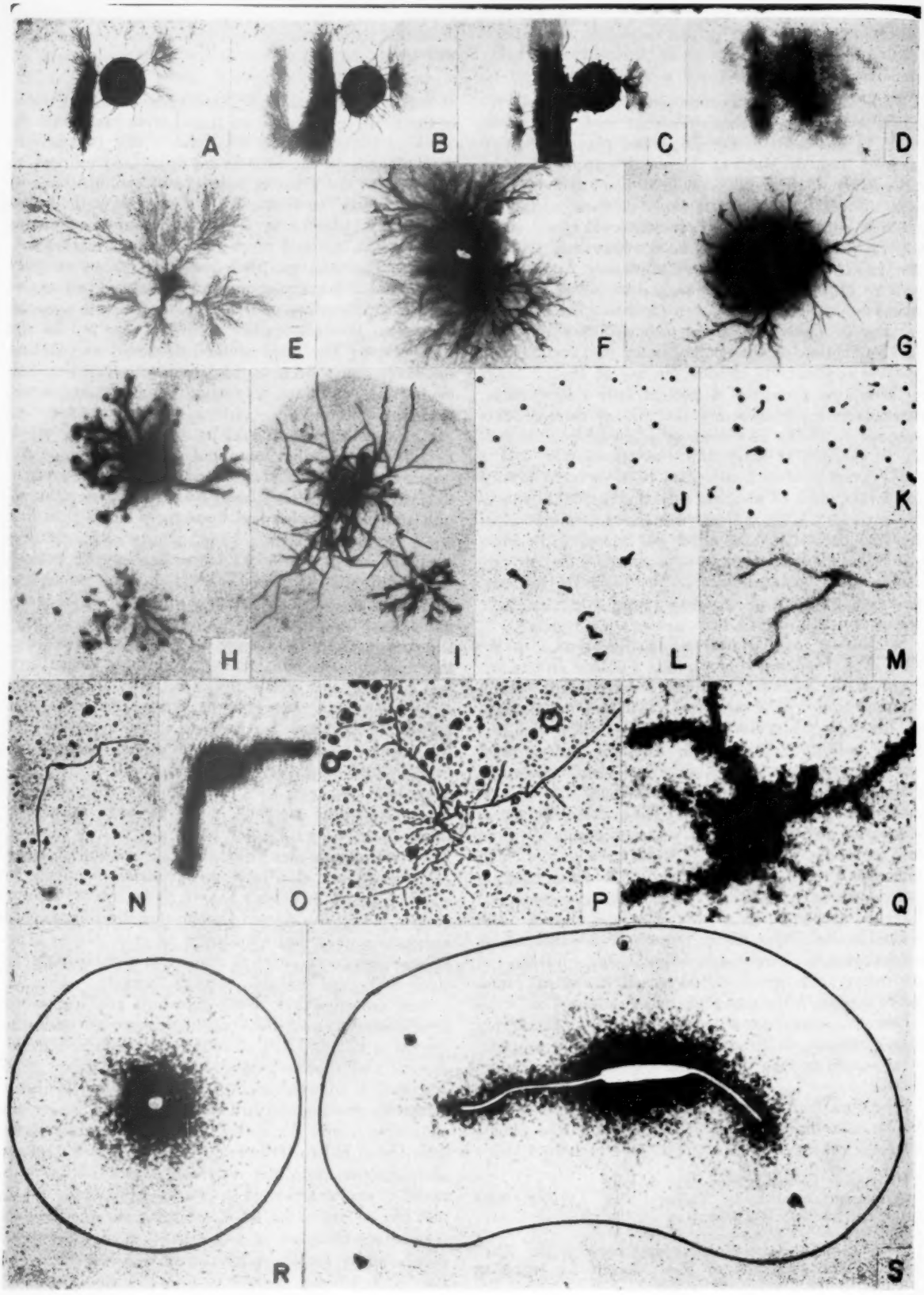
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of nonradioactive medium to the left of the radioactive drop were inadvertently placed in contact at 1 point and that the fungus grew around, rather than across, the bare glass area). If the areas of bare glass are too narrow, allowing dense growth between the radioactive and nonradioactive media, diffusion of radioactivity occurs even though the humidity is carefully controlled (Fig. 1, C). Furthermore, fungi such as *H. victoriae*, which grow rapidly and produce much aerial mycelium, give blurred autoradiographic images when tagged by this method. (Fig. 1, D).

Autoradiographs of cultures of the blackpatch fungus grown on nonradioactive media from small fragments of tagged, washed mats of mycelium are shown in Fig. 1, E-I. Fig. 1, E was made from a culture killed and autoradiographed after 2 days growth on oatmeal agar and shows no evidence of diffusion of radioactivity from the tagged mycelium into the surrounding medium. Fig. 1, F and G represent cultures killed after 12 day growth periods on 1/10 strength oatmeal and water agar respectively. Here it is obvious, especially in Fig. 1, G, that radioactivity diffused into the medium. Autoradiographs of similar cultures of the other 4 species of fungi showed the same distributions of radioactivity. In no case was evidence of diffusion of radioactivity from tagged, washed mycelium found in cultures grown for 3 days or less on nonradioactive media but diffusion was observed in older cultures which had either ceased to grow or were growing very slowly. Diffusion of radioactivity in old cultures, probably as a result of autolysis of some of the cells, complicated attempts to study the transfer of radioactivity through hyphal anastomoses. Fig 1, H and I represent the results of 1 attempt to follow such movement. Here, fragments of tagged mycelium were planted on drops of water agar at points 4-5 mm. from similar plantings of nonradioactive mycelium. Growth was very slow and no fusions of the 2 mycelia occurred until 10-15 days had elapsed. The cultures shown in Fig. 1, H and I were 12 days old when killed and the autoradiographs showed that the entire drop had become slightly radioactive as a result of diffusion from the tagged mycelium. Moreover, even in the absence of any physical contact between the 2 mycelia (Fig. 1, H) the nontagged mycelium became radioactive (apparently as a result of absorption and concentration of the radioactivity of the medium) although much less intensely so than in cases in which fusion of the 2 mycelia occurred (Fig. 1, I).

Autoradiographs of tagged, washed spores are shown in Fig. 1, J-M, O, Q-S. Fig. 1, N and P are photographs of the dried cultures from which the autoradiographs shown in Fig 1, O and Q were made and represent, respectively, 5 and 24 hr. growth from single spores. These figures indicate that radiocarbon was evenly distributed in the hyphae produced following germination of tagged spores. The faintness of the autoradiographic image in Fig. 1, Q, lower left, apparently resulted from screening out of part of the emitted beta particles by the dried agar medium since the hyphae grew under the surface of the medium in this area (Fig. 1, P).

All the autoradiographs shown except Fig. 1, R, S were made with Eastman Kodak No-Screen X-ray emulsion. With this emulsion, considerable background fog accumulates during long exposure periods (Fig. 1, M, Q) making determination of the precise limits of the autoradiographic images difficult or impossible. Nuclear track emulsions are more suitable for such determinations since they accumulate background fog at a much slower rate (Fig. 1, R, S). Since the maximum range in nuclear track emulsions of the beta particles emitted by C^{14} is approximately 90μ, the autoradiographic images are limited to an area extending a distance of 90μ from the tagged spore or germ tube unless some of the C^{14} escapes from these structures. In Fig. 1, R and S, camera lucida drawings of a nongerminated spore of *B. cinerea* and a germinated *H. victoriae* spore have been superimposed upon the autoradiographic images which are encircled by black lines representing a 90μ distance from the tagged organism. The exposure periods used for Fig. 1, R and S were, respectively, 5 times and 2 times those required to produce satisfactory autoradiographic images. Since the images produced were well within the 90μ limit, it is apparent that the C^{14} either remained within the tagged structures or escaped in quantities too small to be detected by this method. In order to test the possibility that C^{14} escaped from the spores and was removed by the washing process, spores produced by *B. cinerea* and *H. victoriae* on aerial mycelium were picked from the radioactive drops with a fine steel needle (care being taken not to allow the spores to come in contact with the radioactive drop during the operation) and transferred directly, without washing, to nonradioactive media. These were killed and autoradiographed at intervals of 2, 4, 8, 16, and 30 min. and 1, 2, 4, 8, 16 and 24 hr. after isolation. Measurements of the autoradiographic images yielded no evi-

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FIG. 1. Autoradiographs of tagged spores and mycelium. A-D. Fungi planted on radioactive drops and allowed to grow across bare glass areas onto strips of nonradioactive medium. A-C. Blackpatch fungus. D. *H. victoriae*. E-I. Cultures of the blackpatch fungus grown on various media from bits of tagged, washed mycelium: in H and I, tagged mycelia were planted at upper center and nonradioactive mycelia were planted at lower center in H and lower right in I; arrows in I indicate hyphal fusions. J. *G. cingulata* spores, not germinated. K. *C. falcatum* spores some of which have germinated. L. *B. cinerea* spores, germinated. M-Q. Growth produced by single tagged *H. victoriae* spores: N and P are photographs of the dried cultures from which the autoradiographs O and Q were made. R, S. Camera lucida drawings of a nongerminated *B. cinerea* spore and a germinated *H. victoriae* spore superimposed on the autoradiographic image of each. (See text for further explanation.) Magnifications: A-D natural size; E-G x 2; H, 1 x 5; J-M x 8; N-Q x 50; R, S x 300. Autoradiographic exposure periods: A-D 5 days; E-I 30 days; J-L 15 days; M, O 35 days; Q 100 days R, S 75 days. Activity of medium used for tagging: 30 microcuries/ml.

dence of diffusion of radiocarbon from the spores or germ tubes prior to, during, or after spore germination.

None of the control preparations, which were similar to those used in the experiments described above except for being grown exclusively on nonradioactive media, caused detectable fogging of emulsions when exposed for periods equal to those used for making the autoradiographs.

GROWTH, GENETICS, AND PATHOGENICITY OF TAGGED FUNGI.—All of the species used in this study grew satisfactorily on media having activities ranging from 2.5 to 30 microcuries/ml. With the exception of 1 species, *G. cingulata*, which appeared to be slightly retarded at activities greater than 10 microcuries/ml., no effects upon growth rate, sporulation, or morphology which could be attributed to radioactivity were observed in cultures grown on radioactive media for 3-7 days. However, slight morphological or physiological effects probably would not be detected with the micro-culture methods used.

To determine if lethal or mutagenic effects were produced as a result of the tagging process, single spores of the 4 sporulating species used were planted on drops of radioactive media (activity 30 microcuries/ml.), with similar plantings on nonradioactive drops serving as controls. The spores produced were harvested after 7 days, washed, and approximately 5000 tagged spores and an equal number of non-tagged spores of each species were plated on potato-dextrose agar. No decrease in viability or increase in variability resulting from the tagging process was detected when the colonies produced were examined and counted. Tagged spores of *H. victoriae* and *G. cingulata* incited disease symptoms indistinguishable from those produced when nonradioactive spores were used as the source of inocula.

Lethal and mutagenic effects were obtained by allowing cultures to remain on radioactive media for periods much longer than those used for tagging purposes. Single spores of *H. victoriae*, *B. cinerea*, and *G. cingulata* were planted on radioactive and nonradioactive drops of media and the spores produced were harvested after 10, 20, 40, and 60 days. The fraction of tagged spores (expressed in per cent of controls) capable of producing visible colonies when plated on potato-dextrose agar after each interval was obtained by averaging the results of 3 separate tests. The results presented in Fig. 2 show that the viability of the spores of all 3 species decreased as the period of exposure to the radioactive medium increased. The large standard errors obtained in some of the tests (Fig. 2), probably as a result of uncontrolled variables involved in the micro-culture methods used, indicate that these results are not sufficiently accurate to provide precise estimates of the lethal effects produced by exposure to radiocarbon. At the same time, it seems safe to conclude that media having activities several times greater than any employed in this study may be used successfully for tagging fungi.

Further tests of mutagenic effects were carried out

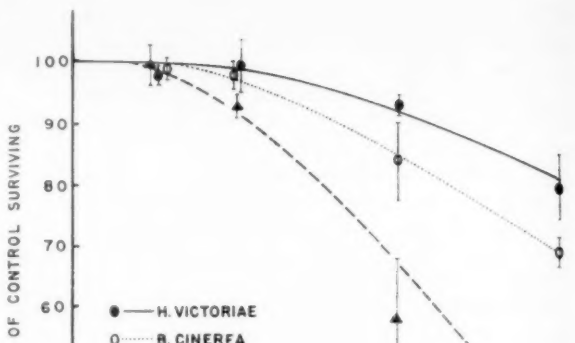

FIG. 2. Survival of spores of 3 species of fungi after exposure to media containing radiocarbon and having an activity of 30 microcuries/ml.

with only 1 species, *G. cingulata*. Single spores from a clumped-conidial culture of this fungus (8) were planted and allowed to remain on drops of medium having an activity of 30 microcuries/ml. for a period of 60 days. The spores were then harvested, plated on oatmeal medium, and the resulting colonies examined for morphologically distinct mutant types. A total of 48 mutants were found among 8,190 colonies produced by the surviving spores. Elimination of 9 of these mutants, which represented duplications and presumably resulted from multiplication of single original mutants, gave a ratio of 1:210 as the minimum frequency of mutation. This was approximately 5 times the frequency (1:991) obtained from control cultures. Since approximately 40 per cent of the spores produced on radioactive medium survived (Fig. 2), the 39 non-duplicated mutants found in the surviving fraction represented nearly twice as many mutants as would be expected to occur spontaneously in the surviving and non-surviving fractions combined. Five mutants, selected at random from those produced by exposure to radiocarbon, were crossed with wild-type cultures by *G. cingulata*. The segregations obtained from these crosses indicated that each of the mutant characters was controlled by a single genetic factor. One of these mutant characters was of considerable interest since it determined the production of dwarf ascospores, approximately 1/3 normal size. Results of a genetic and cytological study of this mutant will be reported elsewhere.

DISCUSSION.—The demonstration, in this study, that fungi can be tagged effectively by a period of growth on media containing radiocarbon and that subsequent development of the tagged organisms can be followed autoradiographically, makes available to mycologists and plant pathologists an additional research tool

which may prove useful. Since lethal and mutagenic effects were obtained only after long periods of exposure to radioactive media, it seems unlikely that such effects will prevent the use of tagged fungi for plant pathological investigations. The fact that little or no diffusion of C^{14} from tagged spores or germ tubes was found to occur, enhances the possibility of detecting extracellular enzyme activity or toxin production in infection studies with tagged plant pathogens. The techniques developed in this study have involved the use of only 1 of the many radioisotopes available and only a single method for detection of radioactivity. It seems certain, for some problems, that other radioisotopes and methods will prove more suitable. Results obtained with tritium (2) indicate that this isotope may be particularly useful for certain types of investigations involving the use of tagged microorganisms.

SUMMARY

Fungal spores and mycelium were tagged effectively by a period of growth on a medium containing radiocarbon in the form of C^{14}-labeled sucrose. The tagged organisms were assayed for radioactivity and their development followed by autoradiography. The growth characteristics of 5 species of fungi, cultured on media having activities ranging from 2.5-30 microcuries/ml., remained unchanged except for a slight retardation of growth of 1 species at activities above 10 microcuries/ml. Cultures of 4 species, grown on media containing radiocarbon for the 5-7 day period normally used for tagging purposes, exhibited no lethal or mutagenic effects, but such effects were produced after much longer periods of growth on radioactive media. Tagged spores were thoroughly washed, transferred to non-radioactive media for periods ranging from a few min. to 48 hr., after which the cultures were killed, dried, and autoradiographed. Measurements of the autoradiographic images yielded no evidence of diffusion of C^{14} from the tagged spores or germ tubes into the surrounding medium prior to, during, or immediately following spore germination.

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THE ASCIGEROUS STAGE OF THE OAK WILT FUNGUS[1]

T. W. Bretz

Since the report in 1942 (8) that oak wilt is caused by a fungus, and the subsequent description of the fungus, *Chalara quercina* Henry, in 1944 (5), the only described method of reproduction for the species has been by asexual endoconidia. The existence of the sexual or perfect stage of the fungus was recently discovered in culture (1).

When compatible isolates of *Chalara quercina* are grown together on suitable culture media, fertile perithecia develop in a rather distinct zone where the growths of the 2 isolates merge. Since fertile perithecia were not obtained when these isolates were grown alone, it was postulated that the fungus is heterothallic in character. This has since been substantiated by Hepting et al (6) who also report that the species consists of self-sterile, hermaphroditic strains. Another hitherto undescribed spore form has been found in cultures producing fertile perithecia (2). These are asexual, thick-walled, olivaceous to brown spores of variable shape and size, formed endogenously and intercalarily in hyphae, which may also produce the typical hyaline endoconidia of *C. quercina*. They appear to be well suited to survival under unfavorable environmental conditions and are thought to function as resting spores.

The connection between the perithecial stage and the asexual stage of *Chalara quercina* was established as follows. Single-ascospore isolations consistently resulted in the recovery of typical *C. quercina* cultures. Microscopic observations revealed that the ascospores, upon germination, give rise to characteristic *C. quercina* endoconidia. Typical oak wilt symptoms developed in susceptible species inoculated with ascospores and single-ascospore isolates, from which *C. quercina* was subsequently recovered.

The morphological characteristics of the perithecia are such that the fungus was tentatively assigned to

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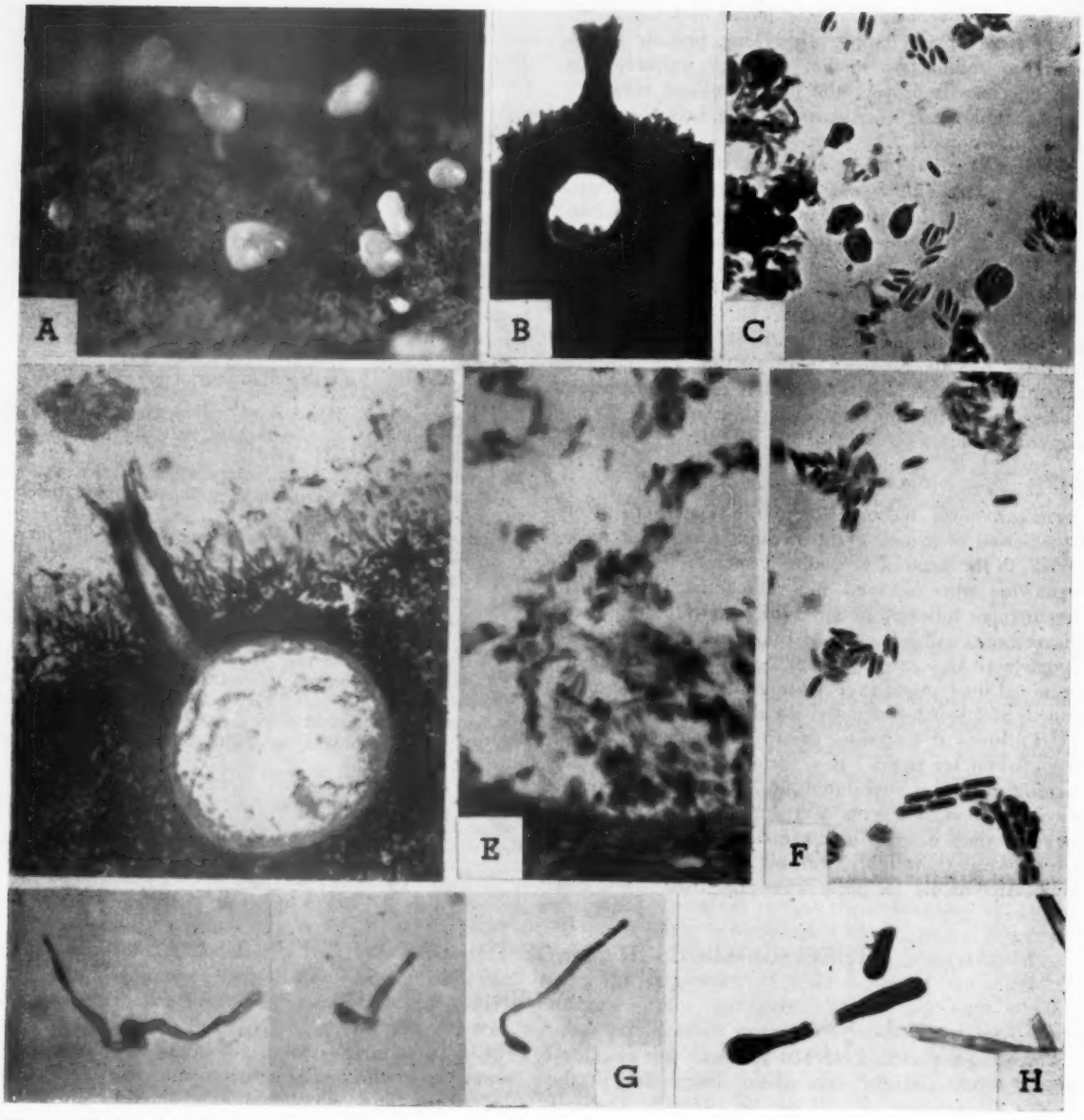

FIG. 1. *Endoconidiophora fagacearum* Bretz sp. nov. A) Fertile perithecia in culture, 25×; B) free-hand section through perithecium, 50×; C) asci and ascospores, fresh mount, 680×; D) median, histological section through perithecium, 100×; E) differentiated asci within perithecium, 680×; F) ascospores and endoconidia, 680×; G) germinating ascospores, 680×; H) thick-walled, asexual resting spores, 680×.

the genus *Ceratostomella* (2). Davidson (4), however, is of the opinion that our present knowledge of the *Ceratostomella* complex indicates that conidial stages are much more reliable for placing the species in their natural groups. He separated the endoconidial group (3), to which this fungus belongs, and placed it in the genus *Endoconidiophora*, established by Münch (7). In the belief that the use of this generic designation will result in less confusion than any other generic name available, the writer is assigning the fungus to the genus *Endoconidiophora*. Because the fungus is pathogenic to species in several genera of the Fagaceae, the name *Endoconidiophora fagacearum* is proposed.

TECHNICAL DESCRIPTION.[2]—*Endoconidiophora fagacearum* Bretz sp. nov.

Conidial stage: *Chalara quercina* Henry (5).

Perithecia[3] begin to form in 7 to 10 days after

[2] The Latin description was prepared by Edith K. Cash, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

[3] Description based on perithecia obtained in culture.

inoculation of culture dishes with compatible isolates; occur singly or in groups; black, flask-shaped, inconspicuous, with spheriodal base 240-380 μ in diameter almost wholly embedded in the subiculum; walls membranous to leathery; ordinarily only 1, rarely 2, erect beaks, 250-450 μ long, black, terminated by a cluster or fringe of hyaline filaments; asci globose to subglobose, 7-10 μ in greatest diameter, evanescent, 8-spored; ascospores hyaline, 1-celled, elliptical and slightly curved, 2-3 by 5-10 μ, collecting at the ostiole in a sticky, creamy-white mass that does not disintegrate in water, upon germination at 25° C. producing hyaline endoconidia; asexual, thick-walled, olivaceous to brown, polymorphic spores, 3.5 to 5.5 μ wide to 5 to 20 μ long, formed endogenously and intercalarily in hyphae which may also produce hyaline endoconidia, occur in perithecium forming cultures. Heterothallic.

Hosts: *Quercus* spp., *Castanea mollissima, C. sativa, Lithocarpus densiflorus* and *Castanopsis sempervirens.*

Type, For. Path. 97476, perithecia produced in culture, deposited in the Mycological Collections of the Bureau of Plant Industry, Soils and Agricultural Engineering.

Endoconidiophora fagacearum Bretz sp. nov.

Perithecia singula vel aggregata, atra, lageniformia, basi sphaerioidea 240-380 μ in diam. in subiculo immersa, pariete membranaceo vel coriaceo, rostris 1-2, rectis, 250-450 μ longis, atris, apice fibris hyalinis fimbriatis; asci globosi usque subglobosi, 7-10 μ in diam., evanescentes, octospori; ascosporae hyalinae, unicellulares, ellipticae, subcurvatae, 2-3 $\times$ 5-10 μ, in massam mucosam cremeam circum ostiolem agglutinatae, germinantes et endoconidia hyalina gerentes; -sporae agamicae polymorphicae, crassi-tunicatae, olivaceae usque brunneae, 3.5-5.5 μ latae 5-20 μ longae, endogene et intercalariter in hyphis interdum etiam endoconidia hyalina gerentibus oriundae.

In culturis e ligno *Quercuum* spp. osilatis evoluta.

Perithecia have been obtained in culture as a result of pairing compatible isolates of the fungus obtained from naturally infected oaks (*Quercus alba* L., *Q. borealis* Michx., *Q. coccinea* Muench., *Q. falcata* Michx., *Q. imbricaria* Michx., *Q. marilandica* Muench., *Q. palustris* Muench., *Q. velutina* Lam.) and Chinese chestnut occurring in Missouri, Arkansas, Ohio, Michigan, Pennsylvania, West Virginia, Kentucky, Tennessee, North Carolina and Virginia. Without magnification the perithecia are not evident in culture until the ascospores are extruded from the ostiole. They are then readily detected by the unaided eye because of the conspicuous, creamy-white, glistening masses of ascospores which collect at the mouth of the perithecial beak (Fig. 1, A). The cluster of hyaline filaments that terminate the perithecial neck (Fig. 1, B) appear to be so arranged that they form a funnel-like structure into which the ascospore masses emerge. The asci appear to arise from a layer of tissue that lines the cavity of the perithecium. Because of their ephemeral nature, spore-bearing asci are difficult to find. However, an occasional microscopic amount of immature perithecia will contain a few recognizable, differentiated, asci (Fig. 1, C). In transverse sections of asci it is not uncommon to find a grouping of 8 spores (Fig. 1, E), which is believed to be the usual number of spores per ascus. Apparently the ascospores are liberated from the asci within the perithecium almost immediately after differentiation. They are readily distinguishable from the endoconidia (Fig. 1, F) and germinate rapidly at 25° C. in 2 per cent dextrose solution, the single germ tube giving rise to endoconidia (Fig. 1, G).

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SEXUALITY IN THE OAK WILT FUNGUS[1]

George H. Hepting, E. Richard Toole, and John S. Boyce, Jr.

In the course of surveys for oak wilt in the southern Appalachian region, the causal fungus was isolated from 29 trees, representing 28 separate infection areas. In the past, this fungus has been known under the name *Chalara quercina* Henry. Recently, however, Bretz found the perfect stage (1), and has named it *Endoconidiophora fagacearum* (2). He obtained fertile perithecia by pairing certain isolates, and produced typical wilt symptoms in susceptible species inoculated directly with ascospores (1, 2).

The authors decided to determine in which trees the composition of the fungus might be such that perithecia could be produced and from which trees pairing of the fungus was required for perithecial formation. In preliminary trials, several Appalachian tissue isolates produced perithecia when suitably paired, and in the case of 1 tree, tissue isolates produced them without pairing (6).

The ascospores are embedded in a sticky mucoid matrix that is sparingly soluble in water. These spores are thus well equipped to adhere to insects and birds and could be a factor in spread over both long and short distances. In the case of other members of the genera *Ceratostomella* and *Endoconidiophora*, insects have been found to be major agents in spread (10, 11, 12, 15).

The first work was done with tissue cultures, so that the behavior of the fungus could be observed as it occurred in trees, and later with single-spore cultures, which provided the pure lines essential for critical studies in fertilization.[2] A preliminary announcement was made of these results (7).

METHODS.—Different isolates of the oak wilt fungus were paired or spermatized[3] on several media. Acorn, casein hydrolysate,[4] chestnut (1), and potato-dextrose agars were used. In preparing the acorn and chestnut agars, 200 gm. of shelled meats were boiled in 500 ml. of water for 1 hour. Water was added to the filtered extract, to make 1 liter. In formulating the purée agars the boiled nut meats remaining from the extract preparations were chopped finely, squeezed through cheesecloth by hand, and suspended in 1 liter of water. Other media were made by adding 100 μg. of thiamin chloride to each liter of acorn extract or purée.

In preparing the potato-dextrose agar, 200 gm. of fresh, peeled, diced potatoes were boiled for 1 hour. Water was added to the filtered extract to make 1 liter. Twenty gm. of dextrose and 15 gm. of agar were added per liter before autoclaving at 15 pounds pressure for 20 minutes.

Some perithecia were formed on all the media. However, the media derived from acorn and chestnut meats were markedly superior to the others from the standpoints of mycelial growth and perithecial production. Little difference was noted between the behavior of the fungus on the extract and purée agars. The addition of thiamin did not noticeably increase the number of perithecia.

Thirty cultures of the fungus, isolated from July through September 1951 from the wood of 29 diseased trees in the Appalachian region, provided the material for this study. These isolates, with the exception of numbers 29 and 30, were cultured from different trees and, except where noted, from different stands (Table 1). Two trees that died of oak wilt during the latter part of 1951 were felled in February 1952 for observations on tightness of bark and for isolations from upper parts. Several patches of the oak wilt fungus, up to a few square inches in area in active conidial fruiting condition, were found under the bark and on the surface of the wood at various distances along the trunk up to 30 ft. above ground.

Pairings were conducted with from 2 to 5 isolates per plate. In the latter instances, 1 isolate was placed in the center of the plate and the remaining 4 were placed equidistant from it and from one another around the plate. Perithecia appeared at the inter-

TABLE 1.—*Appalachian tissue isolates of oak wilt fungus studied*

Locality	Culture number
Kentucky	
Greenup Co.	16
North Carolina	
Buncombe Co.	1, 14, 15, 17, 18
Haywood Co.	8, 12, (19, 20, 21),[a] 22, 23
Tennessee	
Cocke Co.	10, 11, 13, 24
Greene Co.	9, 25, (26, 27),[a] 28
Hamilton Co.	2
Jefferson Co.	3, 4, 5
Sullivan Co.	6
Washington Co.	7
Virginia	
Augusta Co.	[b]29, 30

[a] Those in parentheses were from the same stand.
[b] Both cultures were isolated from tree no. 419.

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Pathologists, Division of Forest Pathology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, Asheville, N. C., in cooperation with the Southeastern Forest Experiment Station, U. S. Forest Service.
[2] The authors are grateful to Dr. W. C. Snyder of the University of California for his counsel in the interpretation of the data obtained from mating cultures.
[3] "Spermatizing" refers to the placing of spores from one thallus on mycelium of another thallus for the purpose of fertilization of the female elements. Olson (9) used this method and term in his crossing experiments with *Ceratostomella fimbriata*.
[4] Supplied by Dr. H. L. Barnett, West Virginia University.

TABLE 2.—*Perithecia produced by pairing tissue isolates of the oak wilt fungus*[a]

Compatibility type	Culture numbers	Culture Numbers: Compatibility type A: 1	10	12	14	15	16	Compatibility type B: 26	27
A	1	0	0	–	0	0	0	–	P
	10	0	0	–	–	–	0	–	P
	12	–	–	0	0	0	–	–	P
	14	0	–	0	0	0	–	P	P
	15	0	–	0	0	0	0	P	P
	16	0	0	–	–	0	0	–	P
B	26	–	–	–	P	P	–	0	0
	27	P	P	P	P	P	P	0	0

[a] P = Fertile perithecia formed.
0 = No perithecia formed.
– = Combination not tried.

faces in from 11 to 18 days at laboratory temperatures in diffuse light. As the cultures aged it was not uncommon to observe perithecia several mm. back from the interfaces.

For spermatizing, conidia were suspended in several ml. of sterile water and the suspension was applied by means of a flamed loop to the receptor mycelium of cultures 1 to 2 weeks old. Commonly, loopfuls of conidia were placed on raised mycelium along lines radiating from the center of a plate, so that new as well as older receptive hyphae were spermatized. Fertile perithecia formed in from 2 to 6 days, with most perithecia appearing within 3 days at laboratory temperatures.

Ascospores and conidia were single-spored by means of a dilution plate method. Masses of spores were transferred to a tube containing several ml. of sterile water, the tube was agitated, and the contents poured on a water agar plate. The excess water was poured from the plate and the plate slanted slightly. The following day germinated spores were picked out under a dissecting microscope and transferred to potato-dextrose slants.[5]

Perithecia Produced by Fertilizing Mass Isolates.—The behavior of tissue cultures recently isolated from wilting oak trees was first studied by pairing cultures from certain trees. It was not necessary to make all possible comparisons among the 8 cultures used in this test in order to determine the sex and compatibilities of the isolates (Table 2). In presenting the mating characteristics of this fungus, the authors have adopted the nomenclature of Hansen and Snyder (4). The latter regard compatibility as distinct from sex. On this basis, fertilization will not occur unless compatible thalli are mated, regardless of their sexual constitution.

When certain isolates had grown together, fertile perithecia were formed along the line of junction. This pairing test established that there were either sex differences or different compatibility types in this fungus. It will be demonstrated that the formation of perithecia following crossing was determined by 2 compatibility types, which we designate type A and type B, rather than by sex, since all isolates could act as either males or females. It is shown in Table 2 that the 6 isolates of compatibility type A formed fertile perithecia only when paired with the 2 isolates of type B.

One tissue isolate, from tree no. 419, produced perithecia alone in culture without being paired. Work with additional isolates from this tree, as will be shown later, demonstrated that the tree contained both compatibility types and that the original isolate was apparently a mixture of the 2. Since isolates from this tree represent a special case, they are treated separately, and not included with the presentation of findings for the other isolates.

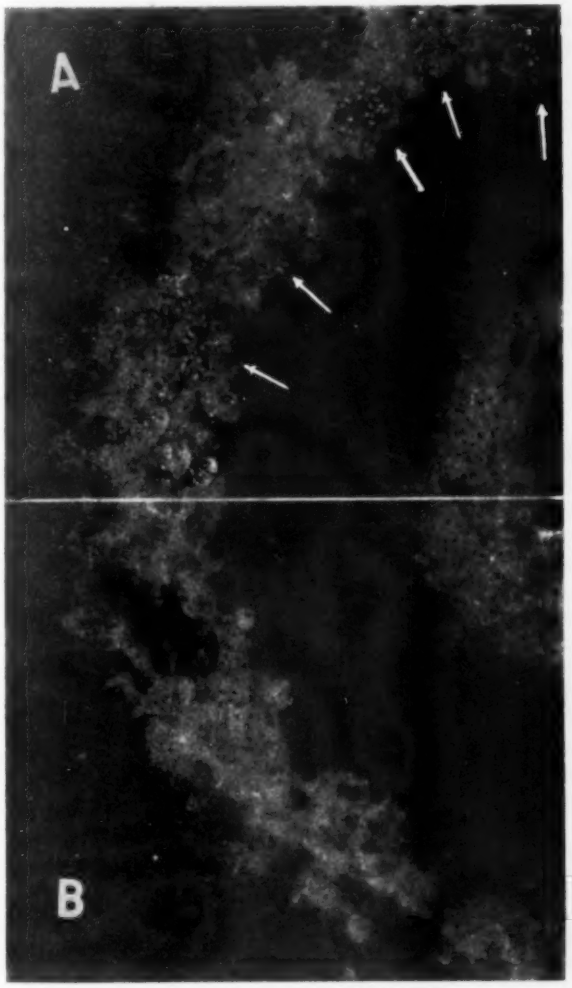

FIG. 1. *A*) Arrows point to groups of perithecia produced in 3 days where loops containing a conidial suspension from a culture of the other compatibility type were applied to a 12-day-old thallus. *B*) No perithecia developed where loops containing conidia of like compatibility type were applied to the same thallus (× 4).

[5] This method was obtained from H. N. Hansen and W. C. Snyder of the University of California.

TABLE 3.—*The production of perithecia by Endoconidiophora fagacearum, following spermatizing of single-spore isolates with conidia*[a]

Compatibility type	Cultures used as receptors	Cultures used as donors: Compatibility type A																Compatibility type B											
		1	2	5	6	7	8	10	11	12	14	15	16	17	18	19	20	3	4	9	13	21	22	23	24	25	26	27	28
A	1	–	0	0	–	–	0	0	–	–	–	–	–	–	–	–	–	P	–	–	–	P	–	–	–	P	–	–	–
	2	–	–	0	0	–	0	0	–	–	–	–	–	–	–	–	0	P	P	–	–	P	–	P	–	P	–	–	–
	5	–	–	–	0	0	0	0	–	–	–	–	–	–	–	0	–	–	–	–	–	P	–	–	–	P	–	–	–
	6	–	–	–	–	0	0	–	–	–	0	–	–	–	–	–	0	–	–	–	–	–	–	–	–	P	–	–	P
	7	–	–	–	–	–	–	0	0	–	–	0	–	0	–	–	–	–	–	–	–	–	–	–	–	P	–	–	P
	8	–	0	0	–	–	–	–	0	0	–	–	0	–	–	0	–	–	–	–	–	–	–	P	P	P	–	–	–
	10	–	0	0	–	–	–	–	–	–	0	0	–	–	–	–	–	–	–	–	–	–	–	P	–	P	P	–	–
	11	–	–	–	–	–	–	–	–	–	–	0	–	–	0	–	–	–	–	–	–	–	P	–	–	P	P	P	–
	12	–	–	–	–	–	–	–	–	–	–	–	0	0	–	–	–	–	–	–	–	–	–	–	–	P	–	–	P
	14	0	–	–	0	–	–	–	–	–	–	–	–	–	0	0	–	P	P	P	P	–	P	–	P	P	P	P	P
	15	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	P	P	–	–	–	–	–	–	–	–	–
	16	–	0	0	–	–	0	0	–	–	–	–	–	–	–	–	–	–	–	P	–	P	–	P	–	P	–	–	P
	17	–	–	0	0	0	–	–	–	–	–	–	–	–	–	–	0	P	P	P	P	P	–	–	–	–	–	–	P
	18	–	–	–	0	0	–	–	–	–	0	–	–	–	–	0	–	–	–	–	–	P	–	–	–	P	–	–	–
	19	–	–	–	–	0	–	0	–	–	0	–	–	–	–	–	0	–	–	–	–	–	–	–	–	P	–	–	P
	20	–	–	–	0	–	–	0	–	–	0	–	–	–	–	0	–	P	P	–	P	–	–	–	P	P	–	–	P
B	3	–	–	–	–	–	–	–	–	–	P	P	–	P	–	–	–	–	0	0	–	–	–	–	–	–	0	–	0
	4	–	–	–	–	–	–	–	–	–	P	P	–	P	–	–	–	–	–	0	0	–	–	–	–	–	0	–	0
	9	–	–	–	–	–	–	–	–	–	P	–	–	P	–	–	–	–	–	–	–	–	–	–	0	0	–	–	0
	13	–	–	–	–	–	–	–	–	–	P	–	–	–	–	–	P	0	–	–	–	–	–	–	–	0	–	–	–
	21	–	–	P	–	P	P	–	–	–	P	–	P	–	–	–	–	–	–	–	–	–	–	0	0	–	–	–	–
	22	–	–	–	–	–	P	P	P	–	P	–	–	–	–	–	P	–	–	–	–	–	–	0	0	0	–	–	–
	23	P	P	P	–	–	–	P	P	–	–	–	P	–	–	–	–	–	–	–	–	–	–	–	0	0	–	–	–
	24	–	–	–	–	–	–	–	P	P	P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0	0	0	–
	25	P	P	P	P	P	P	P	–	P	P	–	P	P	P	P	P	–	–	–	–	–	0	–	–	–	0	0	–
	26	–	–	–	–	P	–	–	–	–	P	P	–	–	–	–	–	0	–	–	–	–	–	–	–	–	–	0	0
	27	–	–	–	–	–	–	–	–	–	P	P	–	–	–	–	–	0	–	–	–	–	–	–	–	–	–	–	0
	28	–	–	–	–	P	–	–	–	–	P	–	–	P	–	–	–	0	0	0	–	–	–	–	–	–	–	–	–

[a] P = Fertile perithecia formed.
0 = No perithecia formed.
– = Combination not tried.

The compatibility types of the remaining Appalachian isolates were determined by pairing each of them with a culture of type A and of type B. These tests established the heterothallic nature of the available oak wilt fungus isolates, and the fact that, except for isolates from tree no. 419, these tissue isolates were pure compatibility types since each formed perithecia when paired with either the A-tester or the B-tester but never with both. These pairings did not throw light on sex versus compatibility. Spermatizing, however, does show the ability of a given culture to act as a female, as a male, or both. A few tissue isolates were cross-spermatized, and crossed cultures produced fertile perithecia where they were of different compatibility types.

Perithecia Produced by Fertilizing Single Conidial Isolates.—Although the performance of the tissue isolates indicated that, except for the isolates from tree no. 419, they were pure lines, single-spore cultures were obtained from all isolates, and the major spermatizing experiments were done with 3 single-spore lines from each tissue isolate. Duplicate cultures were prepared for each of the 3 lines representing each tree. After these had grown for 1 to 2 weeks at room temperature, conidial suspensions were applied to them. Conidia from up to 9 different tree isolates were applied to the 6 cultures representing each tree. It was considered unnecessary to spermatize every isolate with every other isolate (Table 3).

The spermatizing showed that the 3 single-conidial lines from each tissue isolate behaved similarly with respect to compatibility. Single-spore isolates from all trees functioned as males, resulting in fertile perithecia within 2 to 5 days, when conidia from them were applied to cultures of the other compatibility type (Fig. 1). Single-spore isolates from all trees also acted as females, producing fertile perithecia when spermatized with conidia from cultures of the opposite compatibility. These isolates were thus hermaphroditic. Table 3 shows that fertilization could be obtained when any culture of compatibility type A was used as the source of conidia applied to any culture of type B, and vice versa. However, no fertilization was achieved from crosses between cultures of the same compatibility type. The isolates tested are thus shown to be hermaphroditic, self-sterile, interfertile, and of 2 compatibility types.

The behavior of *Endoconidiophora fagacearum* conforms closely to that of *Hypomyces solani* f. *cucurbitae*, as determined by Hansen and Snyder (4, 5) and Hirsch (8). In the case of *H. solani* f. *cucurbitae*, unisexual males and females, and neuters also oc-

curred, although the fungus proved to be typically a self-sterile, interfertile hermaphrodite of 2 compatibilities. Further study of the oak wilt fungus, particularly with older cultures, may reveal the occurrence of these additional forms.

Many factors influence the success of spermatizing. While better perithecial development has been obtained on chestnut and acorn agar than on potato-dextrose, different batches of either vary in suitability. Experiments on the influence of age of receptor thallus showed this factor to be of prime importance. Cultures from 7 to 12 days old, grown at normal room temperatures between 70° and 80° F., generally form perithecia copiously when fertilized. Cultures as old as 3 weeks have in many cases failed entirely to form perithecia. The influence of age of conidia on fertilization has not been studied, but conidia from cultures as old as 3 weeks have been used with a high degree of success. Poor results have followed the application of conidia to parts of cultures lacking aerial mycelium.

COMPATIBILITY CONSTITUTION OF THE FUNGUS AS IT OCCURRED IN TREE NO. 419.—As mentioned earlier, the original tissue isolates from tree no. 419 proved to be self-fertile. To determine the reason for this, cultures were grown from 38 single ascospores, 16 single conidia, and 11 hyphal tips from germinating clumps of ascospores. After several weeks none of these cultures had produced perithecia, indicating that the isolates were not self-fertile. Crosses between thalli derived from single ascospores from a single perithecium yielded fertile perithecia.

To determine whether all tissue isolates from tree no. 419 were self-fertile, additional isolations were made from 3 twigs of each of 7 large branches. Isolation attempts from the trunk failed. Cultures yielding perithecia were again obtained from 2 twigs of each of 2 branches. Cultures from the third twig from these 2 branches and all the cultures from the other 5 branches failed to yield perithecia.

Single-conidial isolates from both the self-fertile and the self-sterile tissue cultures, when cross-spermatized by hermaphrodites of each compatibility, again proved hermaphroditic. The self-sterile isolates all proved to be of compatibility A, whereas the self-fertile isolates proved to be mixtures of types A and B. The original fertile tissue cultures from tree no. 419 were mixtures of thalli of opposite compatibilities, and when these thalli were separated by single-sporing, all were self-sterile.

Apparently the potentiality of the fungus to produce perithecia occurred only in certain parts of tree no. 419 where thalli of both compatibilities were present. The experience with tree no. 419 indicates that in determining the compatibility type or types in a tree, isolations from several parts of the tree may be necessary.

DISCUSSION.—Where cultures of a self-sterile interfertile fungus are simply paired, the formation of perithecia could mean either that the cultures are of different sexes with no compatibility factor present, or that the cultures are of different compatibility types and either both hermaphroditic, one a male and the other a female, or one unisexual and the other a hermaphrodite. All of these possibilities exist in the cases of the pairing experiments with species of *Ceratostomella* reported by Davidson (3), Swingle (14), and Shafer and Liming (13). By spermatizing, however, as performed by Hansen and Snyder (4, 5), Hirsch (8), and Olson (9), male and female capabilities can be determined, and sex separated from compatibility.

Results reported here enable one to "tag" cultures, and this may be very useful in interpreting field observations. Thus, if oak wilt were spread by conidia from an infection center involving the fungus with compatibility A, all additional infections from this center would yield only A isolates. Where the infections in an area contain trees with A thalli and trees with B thalli, such as the Greene County, Tennessee, area, then the infecting spores could have been ascospores from 1 or more perithecia (since the 2 compatibilities probably occur in a 1:1 ratio in the ascus (4)), or conidia from trees containing different compatibility types. Cultures from some of the Appalachian areas, such as those from 5 trees in Asheville, North Carolina, were all of a single compatibility type, while those from some other areas, such as near Waynesville, North Carolina, and Tusculum, Tennessee, proved to be of type A in some trees and type B in others. Excluding isolates from tree no. 419, 16 of the Appalachian isolates were of compatibility A and 12 of B. The possibility remains that the few isolations made from each tree failed to reveal the presence of both compatibility types except in tree no. 419.

SUMMARY

Through cross-fertilization, with conidia as male cells, the oak wilt fungus, as represented by cultures from the southern Appalachian region, was found to be hermaphroditic, self-sterile, and interfertile.

Two compatibility types, herein called A and B, occurred, and interfertility occurred only between paired or spermatized cultures of opposite compatibility type.

The Appalachian isolates represented 28 separate infection areas in North Carolina, Tennessee, Virginia, and Kentucky.

Several tissue isolates from 1 of the trees produced perithecia without crossing. Single-spore isolations from these cultures were all self-sterile, and crossing experiments showed that thalli of both compatibilities occurred in this tree. All other tissue isolates were of a single compatibility.

Since perithecia can be produced and cultures "tagged" as to sex and compatibility within a few days, studies on the role of the perithecial stage in the spread of oak wilt can be facilitated. Conidia, being asexual, can perpetuate only the compatibility type of the thallus producing them. Perithecia, however, yield ascospores of both compatibility types, since

ascospores are the result of the fusion of nuclei of the 2 types.

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A PHYTOPATHOLOGICAL NOTE

A Strain of Cowpea Susceptible to Systemic Infection by Various Strains of Common Cucumber Mosaic Virus.[1] W. H. SILL, JR., and J. C. WALKER.—Most strains of cucumber virus 1 incite local lesions on inoculated unifoliate leaves of cowpea (*Vigna sinensis* Savi.)[2] A notable exception is the strain isolated from common bean (*Phaseolus vulgaris* L.) by Whipple and Walker[3] and designated by them as strain 14. The latter incites no local lesions on inoculated leaves and increases systematically in cowpea to bring about mottling and stunting. In the course of studies involving inoculation of several thousand plants of the Black variety with a local-lesion-inducing strain of the virus, an occasional plant not only failed to show local lesions on the inoculated leaves but developed systemic symptoms. The possibility of contamination of the virus source with strain 14, which is maintained among our stock cultures of cucumber mosaic virus strains, was explored by reinoculation from such occasional systemically-infected plants to other cowpea plants. Typical local lesions and no systemic infection always followed. Inoculation to other test hosts also failed to give evidence of contamination of the virus source. After these observations and tests, any systemically-infected cowpea plants which appeared were grown to maturity. The self progenies of 79 such plants have been tested with the same local-lesion inducing strain of the virus. Every plant from each progeny tested showed systemic symptoms but no local lesions on inoculated leaves. Inoculations with 2 other distinct local-lesion-inducing strains of cucumber virus 1 yielded similar results. Strain 14 also produced systemic infection in these progenies as it does in the entire population of the Black variety. Tobacco necrosis virus and tobacco ringspot virus also incite local lesions on inoculated primary leaves of cowpea. When they were inoculated to plants from the self progenies mentioned above they each incited typical local lesions. The plants in the self progenies were typical of the entire population of Black variety except for their reaction to cucumber virus 1. It is probable that they are derived from a mutation in the genes controlling resistance and susceptibility to systemic infection by the local-lesion strains. The inheritance of the newly-observed character is being studied. The observations recorded here are of interest to those using cowpea as a local-lesion host for cucumber virus 1 and to those concerned in breeding programs with cowpea.—Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

[1] This study was supported by a grant from the Research Committee of the Graduate School, University of Wisconsin.

[2] Sill, W. H., Jr., and J. C. Walker. Cowpea as an assay host for cucumber virus 1. Phytopath. 42: 1952.

[3] Whipple, O. C., and J. C. Walker. Strains of cucumber-mosaic virus pathogenic on bean and pea. Jour. Agr. Res. 62: 27-60. 1941.

YELLOW SPOT, AN ERIOPHYID MITE INJURY ON PEACH [1]

Norton S. Wilson [2] and L. C. Cochran [3]

Spotting and chlorosis of vein-bordering tissue of peach foliage has been reported in several states, and has recently attracted much interest because of similarity of symptoms to those of certain mosaic-type virus diseases. Keifer (5, 6) reported longitudinal rolling and production of minute yellow dots in peach leaves, due to feeding by the eriophyid mite *Vasates cornutus* (Banks).

Vasates cornutus, the peach silver mite, occurs generally throughout North America and is responsible for the well-known silvering of peach foliage. According to Keifer (4), 2 forms of the mite occur, the summer leaf form of both sexes and the hibernating winter form of females only. The mites are very small, averaging about 200 to the in. (Keifer 3, 6), and almost invisible to the unaided eye. They feed on both surfaces of the leaf and hibernate under bud scales.

This paper is a report of investigations to determine whether the yellow spot associated with *Vasates cornutus* is due to injury resulting from toxic substances introduced by the mite or whether the symptoms are due to a virus, perhaps transmitted by the mite.

Description of symptoms.—Typically affected Lovell peach seedlings show light-yellow chlorotic spots and chlorotic areas adjacent to veins. Individual spots range from pinpoint size to more than a mm. in diameter, and are generally circular to irregular in shape (Fig. 1).

At first the spots have diffuse borders, but as they become more fully formed the borders tend to become sharper. In severe cases the spots sometimes coalesce to produce a vivid mottle effect (Fig. 1, B.). In a nursery row of Lovell peach seedlings some are strikingly affected, as compared with others, indicating differential susceptibility to the injury.

Yellow spot has been seen on a number of peach varieties and seedlings of varieties. Salberta and seedlings of Salberta appear to be very strong reactors. On certain seedlings the spots develop red borders and on leaves the centers become necrotic and drop out, producing a shot-hole effect (Fig. 2, A). Spots also appear on the current-season stem growth. At first the spots on stems are obscure and pale yellow, but as they increase in size they become slightly raised and darker. On seedlings showing red color on the mature portions of the stems the spots on the immature portions become reddish purple (Fig. 2, B).

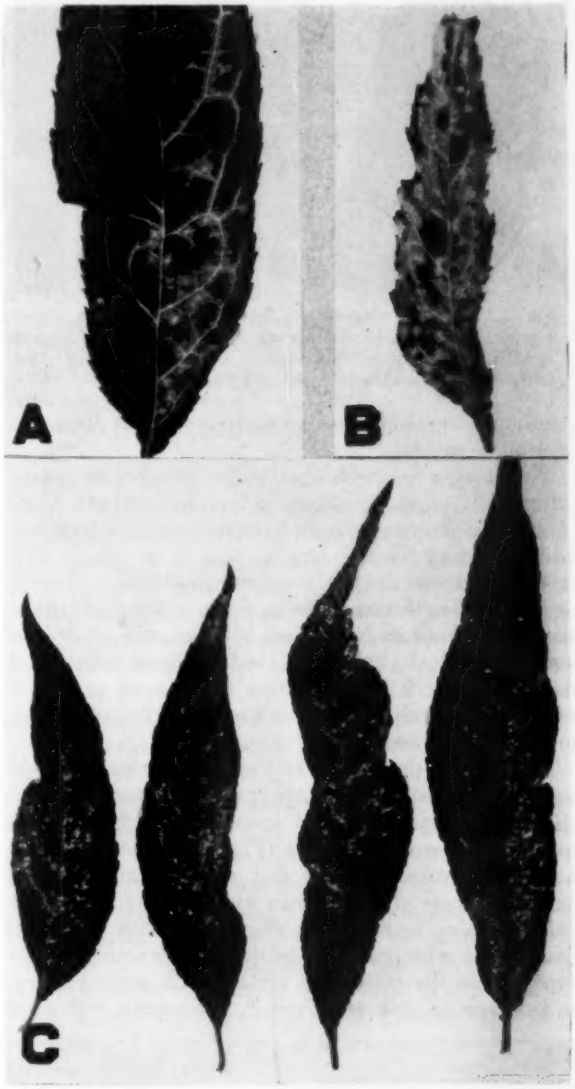

Fig. 1. Lovell peach seedling leaves showing: A, Vein-associated chlorosis characteristic of symptoms produced on young leaves by *Vasates cornutus;* B, diffuse-type mottle, resulting from coalescence of spots; C, patterns of yellow spots, the most common symptom.

Leaves of J. H. Hale and Elberta rarely show the vein-associated chlorosis and vivid spotting found on Lovell seedlings, but both minute yellow spots and larger but rather obscure reddish spots are produced. The center of the latter often drops out to produce shot-holing. Hale leaves often show puckering and longitudinal rolling. On most of the field-grown trees under observation for yellow spot symptoms, silvering of leaf surfaces typical of silver mite infestations developed late in the season, just before leaf drop. This

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The authors are indebted to H. H. Keifer, California State Department of Agriculture, for identification of samples of mites used in this investigation.

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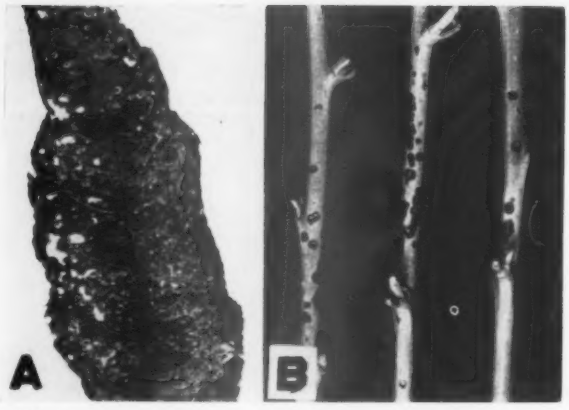

FIG. 2. Leaf and stems of Salberta peach seedling showing mite injury: A, Leaf showing minute spots, the largest of which have formed shot holes; B, current-season stem growth showing various stages of spotting.

symptom is well known and entirely distinct from the spotting symptom.

Yellow spot has been observed on peaches at nearly all periods of the growing season in southern California, but is most noticeable in late summer and fall on new-growth flushes.

ANATOMY OF LEAVES SHOWING SYMPTOMS.—Lovell peach seedling leaves showing various types of symptoms were fixed in Navashin's solution, dehydrated in tertiary butyl alcohol, and imbedded in paraffin. Sections were cut 9, 12, and 15 μ thick, most of which were stained with dilute iron haematoxylin, according to the method reported by Esau (2).

Sections cut through veins having adjacent chlorotic areas and through recently produced yellow spots showed both palisade and spongy parenchyma cells to have been severely altered (Fig. 3). Cell constituents had disintegrated, and the cell often contained a disorganized mass of material in which numerous spherical refractive bodies were dispersed. The refractive bodies and other material in the cells stained intense green when the cells were treated with methyl green in conjunction with acid fuchsin, in contrast with normal cells in which cytoplasmic elements, including plastids, stained red.

Varying amounts of disorganization were found in sections of young leaves that were killed and fixed soon after the mites fed. This disorganization ranged from the disruption of single epidermal cells to groups of several cells and the adjoining palisade cells. These areas are interpreted as representing individual feeding injuries and were the early stages of what later became yellow spot lesions. No feeding sheaths were produced.

This type of disorganization seems to be a common reaction of peach parenchyma cells to certain types of injury and also occurs in senescent leaves. Similarly affected cells have been consistently found in sections of young peach leaves affected with mosaic and also in peach leaves showing spotting symptoms due to ringspot virus. Prolonged feeding of certain species of aphids, particularly those that induce chlorosis and distortion of leaves, produces a similar reaction in peach leaf cells.

In sections through veins with adjacent chlorotic areas numerous parenchyma cells around the vascular bundle were disorganized, as were ray cells in many cases. Occasional hypertrophy of vein parenchyma cells was found (Fig. 3, C). No gumming of xylem vessels was found, and phloem elements did not seem to be affected.

EXPERIMENTAL PRODUCTION OF SYMPTOMS.—During 1950 germinating Lovell peach seeds were planted in flats out of doors. When the seedlings had reached a height of 2 to 3 in. 1 flat containing 15 seedlings (group 1) was exposed for 48 hr. to foliage of J. H. Hale peach which was heavily infested with the mites. Examination of the seedlings at the end of this period showed each to be infested, the mite count ranging from 0 to 15 per leaf. Yellow spotting and vein-associated chlorosis first appeared 12 days later, the affected leaves having from 35 to 197 mites per leaf. The increased number was apparently the result of reproduction. Final readings of group 1, made 32 days after initial infestation, showed that vein-associ-

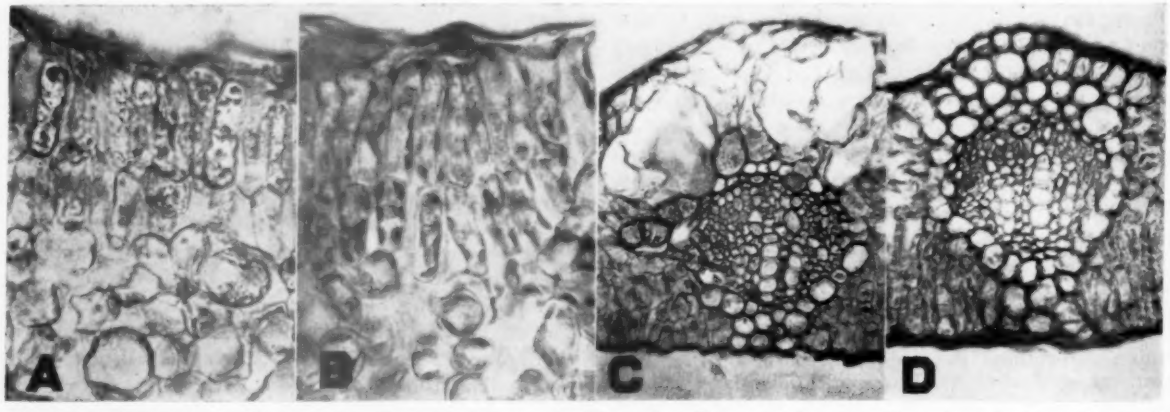

FIG. 3. Transverse sections of Lovell peach leaves: A, Through yellow spot between the veins, showing disorganization of cell contents; B, through corresponding area of normal leaf; C, through leaf vein having chlorotic bordering tissue, showing hypertrophy of vein parenchyma cells; D, through vein of normal leaf.

ated chlorosis, or spotting, was present on 11 of the 15 trees.

Later groups 2 and 3, consisting of 42 8-inch seedlings each, were exposed to infestation for 48 hr., and at the end of that period seedlings of group 3 were given an oil spray. Yellow spotting and vein-associated chlorosis began to appear 10 days later; by the twelfth day symptoms were present on the foliage of 10 trees in each of the groups. Examination of group 3 immediately after the oil spray showed no living mites, and only a few scattered individuals could be found at the end of 12 days. It, therefore, seems probable that the initial 48-hr. feeding was sufficient to produce the symptoms.

Group 4 (42 seedlings) was reserved as a control, and at the time of the final readings 1 leaf on each of 2 trees showed small yellow spots. Examinations of these trees showed that they had been accidentally infested with mites. No mites were found on the remainder of the check trees.

Additional tests were carried out in 1951 in a greenhouse and more adequate provision was made to prevent accidental infestation. Potted Lovell peach seedlings 1 to 4 in. high were divided into 4 groups of 4 trees each, and 3 of the groups were infested by attaching mite-infested peach leaves to each of the test trees for a 24-hr. period. The fourth group was reserved as an uninfested control. Although the infesting material for each seedling carried several hundred mites, only a small percentage of them transferred. Examination of the test-seedling leaves showed from 0 to 60 mites per leaf. Yellow spot symptoms began to appear in some leaves of the test trees as early as 6 days after original infestation, and the average period for symptoms to appear was 9.6 days. Leaves of the check trees developed no symptoms. Results of both series of experiments are in Table 1.

EVIDENCE AGAINST VIRUS AS CAUSATIVE AGENT.—After it was apparent that production of yellow spot symptoms could be definitely associated with the feeding of *Vasates cornutus*, experiments were undertaken to determine whether a virus was involved. Each tree in groups 1 to 3 of the 1951 series was indexed by grafting patches of bark into healthy seedlings. Buds were not used for indexing because of the difficulty of freeing the scales from mites. Before cutting the bark for patch grafts from trees showing yellow spot symptoms, the site of the stem from which the patch was taken was washed with a 2 per cent oil and was examined with a hand lens, to be certain that no mites were present. No symptoms were produced on the index seedling, although similar grafting technique gave excellent transmission of the peach mosaic and ring spot virus diseases. Several of the 1950 seedlings affected with yellow spot were also indexed by patch grafts, with negative results.

To further ascertain that no virus was involved, the following procedure, suggested by Dr. J. M. Wallace, of the University of California Citrus Experiment Station, was followed: approximately 15 to 20 days after infestation, when leaves of groups 1, 2, and 3 of the 1951 series were showing good symptoms, an oil spray was applied to groups 1 and 2. Ten days later apical leaves of trees of group 1 were reinfested with mites, while those of group 2 were maintained without reinfestation. The apical leaves of trees of group 1 developed typical yellow spot symptoms in approximately 10 days. Figure 4, A, shows a reinfested tree of this group with the original yellow spot symptom-bearing leaves at the basal part of the tree, with intervening symptom-free leaves, and leaves at the apex bearing symptoms produced after reinfestation. Group 2, not reinfested, showed only the original symptoms on leaves at the base, with the remainder of the leaves symptom-free (Fig. 4, B). Group 3, on which the mites were not killed, showed yellow spot symptoms on leaves from base to tip.

Production of a second cycle of symptoms following reinfestation on seedlings of group 1 (Fig. 4, A)—as contrasted with group 2, on which the mites had been killed—showed that symptoms were directly associated with the mite feedings. Furthermore, the production of additional symptoms on recovered plants by reinfestation indicated that the symptom-free leaves were not the result of recovery of the tree from virus, but were due to the absence of toxic materials from mite feeding.

FACTORS AFFECTING PRODUCTION OF SYMPTOMS.—Field observations indicate that fully mature leaves of peach do not develop yellow spot following mite infestation. To determine whether this freedom was due to absence of mites on the older leaves or to properties of the leaves themselves, Lovell seedlings 8 to 10 in. high were heavily infested for 24- and 48-hr. periods, after which the mites were killed with an oil spray. Examination of the leaves during the feeding period showed the mites apparently feeding on both young and old leaves. Symptoms developed on the leaves that were rapidly growing and unfolding while the mites were present, but not on the older leaves on which the mites fed, nor on leaves formed after the mites were removed. Feeding periods shorter than 24 hr. were not investigated.

Production of chlorotic areas adjacent to veins ap-

TABLE 1.—*Yellow spot on Lovell peach seedlings 15 days after experimental feedings by Vasates cornutus.*

Group No.	Trees: Number in group	Trees: Number showing symptoms	Number of leaves showing symptoms
1950 Series:			
1	15	6	25
2	42	10	12
3	42	10	22
4 (check)	42	2	2
1951 Series:			
1	4	4	44
2	4	4	15
3	4	4	38
4 (check)	4	0	0

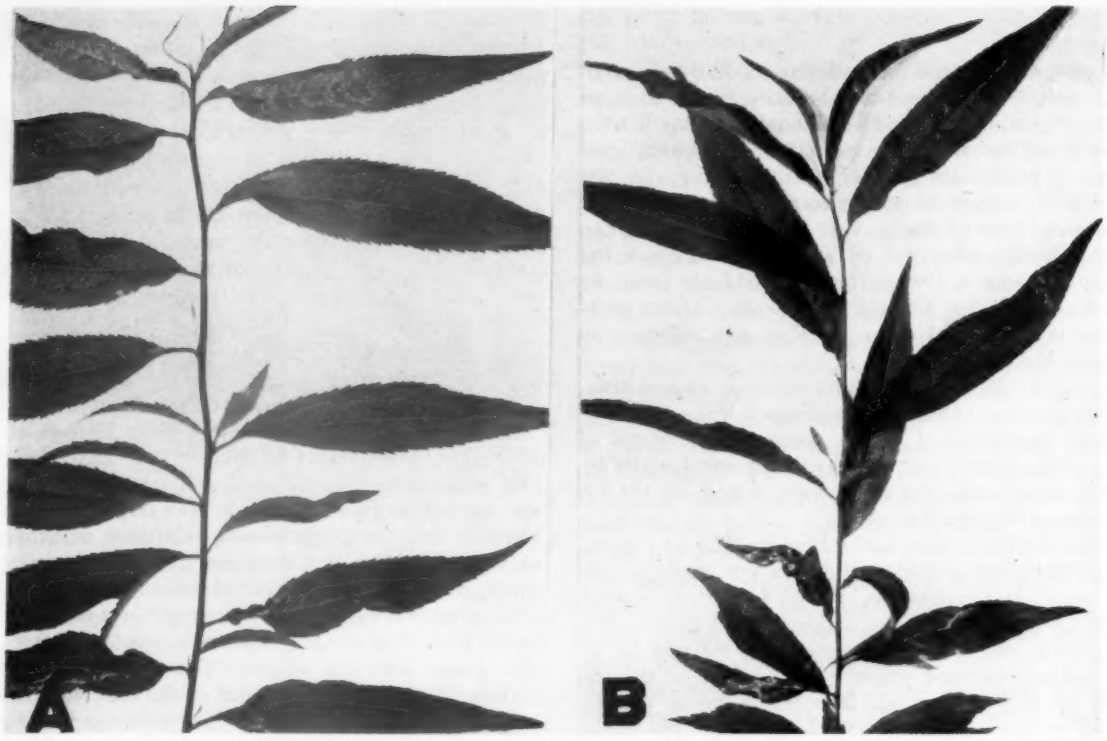

FIG. 4. Peach leaves: showing results of initial feeding by mites: A, Seedling of group 1 with yellow spot symptoms on basal leaves, and also on apical leaves as a result of reinfestation. B, Seedling of group 2 with yellow spot symptoms on basal leaves only.

pears to be correlated with infestation of very young leaves, and may be due to introduction of toxin into the vascular bundles on the young leaves; whereas, on the older leaves increased thickness of the parenchyma layers around the veins limits feeding to the parenchyma. This theory is supported by observations on field-grown trees on which the yellow spot symptom is predominant and vein chlorosis is rare, as compared with greenhouse trees. It may be that the leaves of field-grown trees are more mature before they become heavily infested, as compared with those experimentally infested in the greenhouse. It is not known what effect other factors, particularly prolonged feedings, may have on the development of such symptoms.

Several factors may influence the type and intensity of symptom development. Yellow spots are usually smaller and less striking on J. H. Hale than on Lovell seedlings. On Salberta and seedlings of Salberta many of the yellow spots fall out, producing shot-hole. Some individual Lovell seedlings are more strikingly affected than others. Keifer (5, 6) reported a correlation of mite injury on seedlings having no basal leaf glands, and attributed this effect to interference with mite feeding on young leaves by gland secretions. This belief is supported by the present investigation demonstrating that very young leaves, when fed on by mites, are much more apt to exhibit visible symptoms than are older leaves. Naturally, any factor that would reduce mite feeding on young leaves would influence symptom production. The reaction, however, could also be attributed to some genetic resistance factor correlated with lack of glands.

It seems evident that a toxic material is involved in the production of symptoms and that its effect is localized near feeding points and is not generally systemic, since mites feeding on one leaf do not cause symptoms in mite-free leaves nearby. The material apparently does move short distances within the leaf, both by diffusion among parenchyma cells and along vascular bundles. Thus, *Vasates cornutus* can be considered a toxicogenic mite, according to Carter's definition of the term (1).

SUMMARY

A disorder of peaches, characterized by yellow spotting and vein-associated chlorosis of leaves, longitudinal rolling of leaves, and stem spotting, is described and termed "yellow spot." Sections through symptom-bearing leaves indicate that the parenchyma is primarily affected.

Symptoms were readily produced by experimentally controlled feeding by the mite *Vasates cornutus* (Banks). A 24-hr. feeding period was sufficient to produce symptoms, but the minimum number of mites or the minimum length of the feeding period necessary for symptom production was not determined.

Symptoms were consistently produced experimentally on early-stage leaves, but were not produced on

mature leaves.

After the initial feeding of the mites, a delay of approximately 10 days occurred consistently before visible symptoms appeared. This delay took place, whether the mites were removed after an initial feeding period of 24 to 48 hr. or were allowed to remain on the plant.

Index grafting and other manipulation indicated that no virus was involved.

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A PHYTOPATHOLOGICAL NOTE

A culture plate for agar diffusion assays.[1] J. L. Lockwood, Curt Leben, and G. W. Keitt.—In investigations employing agar diffusion assays for antibiotics and fungicides[2, 3, 4, 5] the limitations of Petri dishes for large numbers of assays and for paper strip chromatogram assay suggested the use of larger culture vessels. Metal-rimmed, glass-bottomed plates, about 25 x 50 cm. and of the type used widely in assaying antibiotics, were expensive and were found to be subject to breakage and leaks, especially when autoclaved. Autoclaving was advisable because the indicator fungus, *Glomerella cingulata* (Stoneman) Spauld. and v. Schrenk, usually grew more slowly than the bacterial contaminants that ordinarily remained in the plates after washing with a germicide (e. g., ethanol). The culture plate that was developed to meet our needs appears to be sufficiently useful and inexpensive to warrant a brief description.

The 3 component parts of the plate, which may be varied in size and composition, are as follows: 1) a rimmed aluminum cooky pan, approximately 1 x 25 x 35 cm., 2) a piece of 1/8 inch plate glass to fit snugly in the bottom of the pan, and 3) a cover approximately 29 x 39 cm. made from 1/8 in. asbestos board (e. g., Transite or Flexboard). Asbestos board is preferable to glass or metal for the cover because the condensate is absorbed.

In setting up the plate, the assembled components were autoclaved and cooled. A small amount of hot 4 per cent agar was pipetted around the edges of the glass to form a seal between the pan and the glass. When the seal had hardened, the plate was placed on a level table and 250 ml. of seeded assay agar at approximately 40° C. added and allowed to harden. The test substances, in paper disks or on seeds, were then applied with provisions for suitable controls on each plate. For our purposes a separate base layer and seed layer were not necessary.

At the close of the incubation period (24-40 hrs.), the agar was loosened from the sides of the pan with a knife, the glass bearing the agar removed, and the measurement of the inhibition zones made in the usual way.

Figure 1 illustrates the components of the plate and the inhibition zones produced by oat seeds treated with ethyl mercury *p*-toluene sulfonanilide (*Ceresan M*).—Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

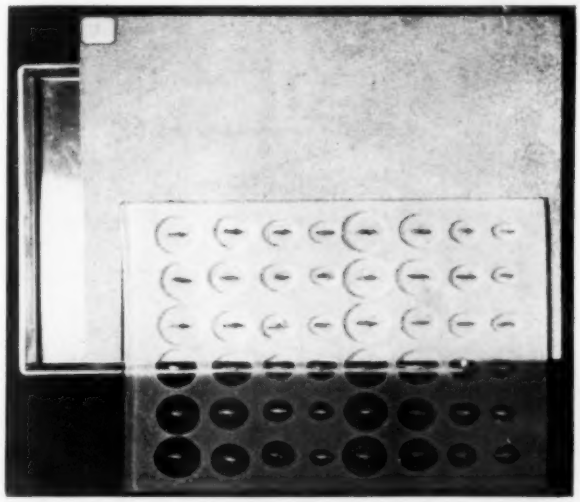

Fig. 1.—The component parts of the culture plate: the aluminum pan, the asbestos board cover, and the glass bearing a culture of *Glomerella cingulata*. The inhibition zones were produced by oat seeds treated with *Ceresan M* at 1.0, 0.5, 0.25, or 0.125 oz. per bu.

[1] Supported in part by the Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation. Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

The authors wish to acknowledge the technical assistance of Gaylord M. Barlow.

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RELATION OF CERTAIN ENVIRONAL FACTORS TO INITIATION AND DEVELOPMENT OF THE WHITE RUST DISEASE OF SPINACH [1]

Robert D. Raabe and Glenn S. Pound

White rust (*Albugo occidentalis* Wilson) of spinach (*Spinacia oleracea* L.) first became important in the Winter Garden region of Texas in 1937 (5, 6). Since that time, the disease has spread to other spinach-growing areas of Texas and the lower Arkansas River Valley. Frequently it has caused heavy losses in Texas. Although inoculum has been introduced to all sections of the country on fresh market spinach, the disease has never become established outside the southwest. This paper reports studies of the effect of certain environal factors on initiation and development of the disease. A preliminary report has already been made (4).

EXPERIMENTAL RESULTS.—*Symptoms.*—Symptoms of the disease are similar to those of the other white rust diseases, except in that some phases they are more pronounced. They first appear as small chlorotic areas on upper and lower surfaces of a leaf. As these areas enlarge, the chlorotic condition becomes more pronounced on the upper surface while the conidial pustules begin to appear as small whitish areas on the lower surface.

Later, the pustules or sori have a white, glassy appearance and look much like yeast colonies on the leaf; they are blister-like, oval, irregularly oval, or elongated, varying in size from about ½ to 2 mm. in diameter and 3 to 4 mm. in length. Frequently, the pustules may be so abundant that nearly the entire under surface of a leaf is covered (Fig. 1, A). Although the pustules are usually borne on the under surfaces of leaves, they are occasionally produced on the upper surfaces, on petioles, branches, and fruit coats.

The disease differs from the other white rust diseases in that oospore formation creates prominent symptoms, especially under greenhouse conditions; oospores are formed in leaves, petioles, main stem, side branches and fruit coats often in such numbers that infected tissues are nearly black (Fig. 1). Although the seed clusters may be thoroughly overrun by the fungus, and both conidia and oospores are produced on the fruit

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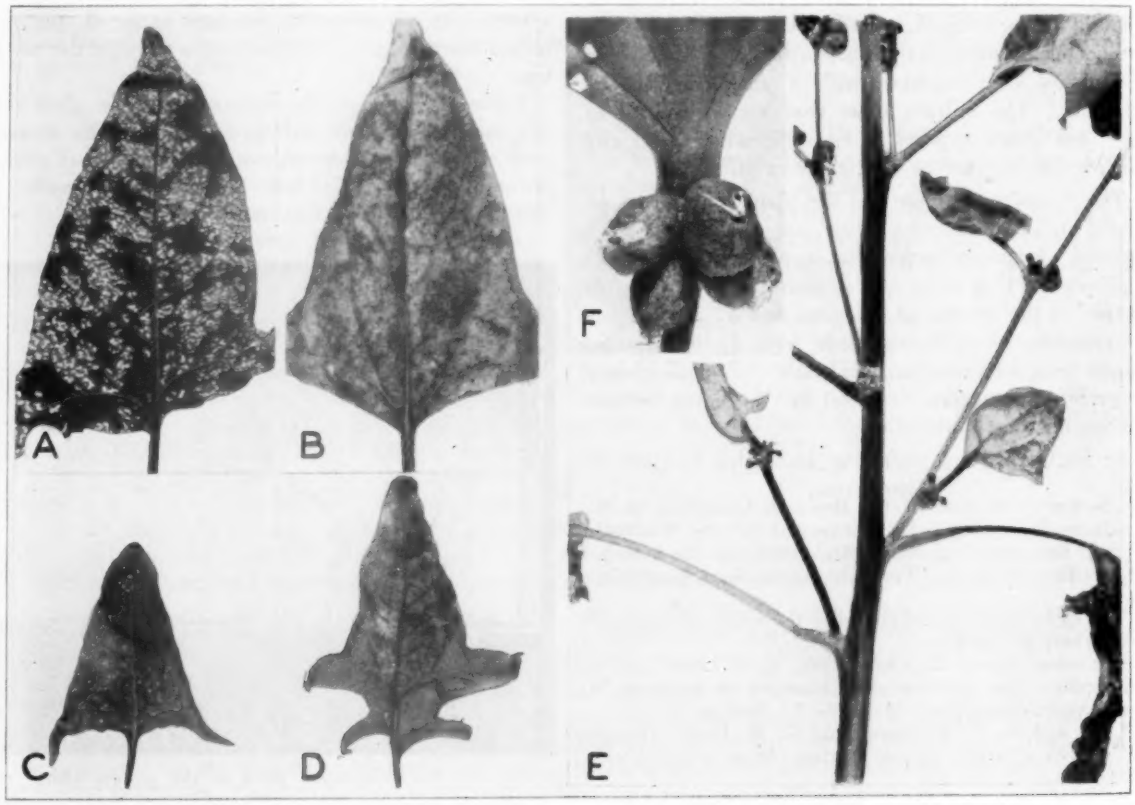

FIG. 1. Symptoms of white rust on spinach. A) conidial sori on vegetative leaf; B) oospore development on vegetative leaf; C) conidial development common at 16° C.; D) oospore development common at 28° C.; E) profuse development of oospores in systemically-infected stem of seed plant; F) oospore and conidial development on seed ball.

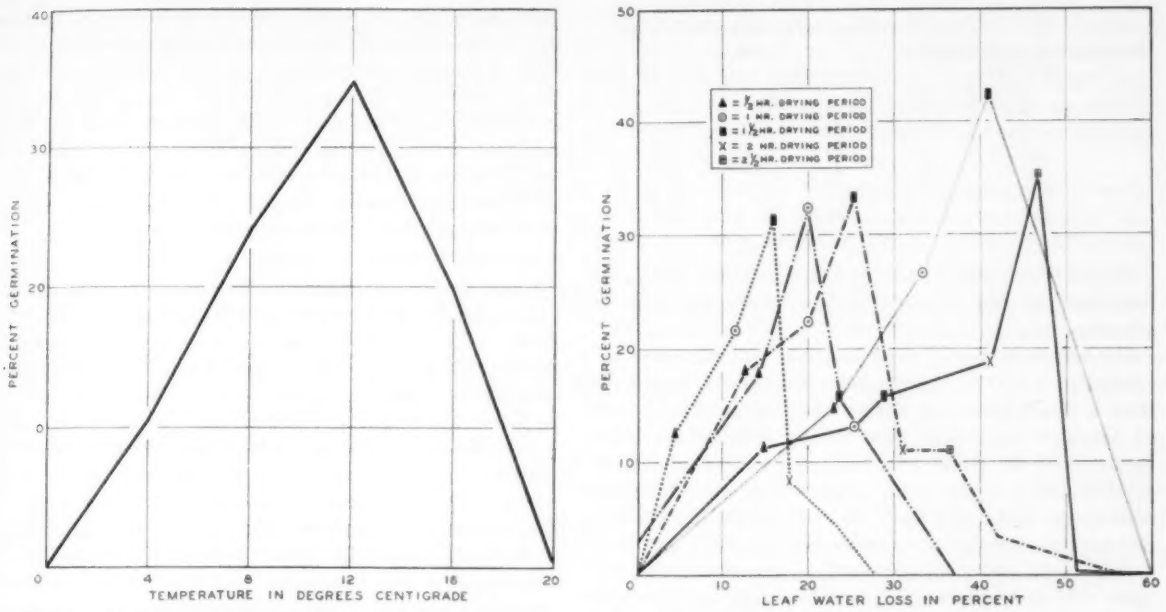

FIG. 2. *Left,* Effect of temperature on the germination of conidia of *Albugo occidentalis. Right,* Effect of water loss upon the germination of conidia of *Albugo occidentalis.* Each curve represents 1 leaf and a separate experiment.

coats, seed from them have always given rise to healthy seedlings.

The fungus is often systemic in plants which have bolted to seed. Only rarely does it become systemic in vegetative plants and develop along with the plant as it bolts. When systemic, it produces both conidia and oospores on all infected parts of the plant. It may cause a slight twisting of the stem and leaves, but there is little or no hypertrophy or hyperplasia as in some of the other white rust diseases.

Frequently the fungus causes necrotic areas in leaves and when infection is severe whole leaves may be killed. It is not uncommon for a severely-affected field to have a brownish cast due to dead leaves.

Host Range.—In a host range study, *Chenopodium capitatum* (L.) Asch., *C. album* L., *C. botrys,* L., *C. hybridum* L., *Kochia scoparia* (L.) Schrad., *Atriplex hortensis* L., *Beta vulgaris* L., (sugar beet, red beet, and swiss chard), *Armoracia rusticana* Gaertn., Mey. and Scherb. (horseradish), *Brassica oleracea* var. *capitata* L. (cabbage) and several species of *Amaranthus* were inoculated. Of these only *C. capitatum* became infected. In addition, all commercial varieties and 89 foreign introductions of spinach were inoculated and found to be susceptible.

Factors Affecting Conidial Germination.—In order to ascertain the effects of environment on initiation and spread of the disease, spore germination tests were made in the laboratory. Conidia for the tests were collected in vials containing a small amount of water. A drop of the suspension was then placed on a cover slip which was inverted over a deep well slide. The cover slip was sealed to the slide with water. The slide was put on a U bend in a Petri dish containing a small amount of water and the dish was then put at the desired temperature. After 48 hours germination counts were made.

It was found that the conidia would not germinate except in the presence of free moisture and that they germinated better in double-distilled water than in any other medium. Other media tested were rain water, lake water, tap water, distilled water, orange juice (0.1, 1.0, 2.0, 4.0 per cent), spinach leaf extracts (0.1, 1.0, 2.0, 100.0 per cent), Czapek's solution (0.1, 1.0 per cent) and 0.01, 0.1, 1.0 per cent solutions of cerelose, glucose, sucrose, sodium chloride, sodium acid phosphate, dibasic sodium phosphate, sodium phosphate, potassium acid phthalate, and sodium acetate.

a) *Temperature* Conidia were placed in temperature chambers at 4°, 8°, 12°, 16°, and 20°C. In all trials the optimum temperature for germination was near 12° C.; germination decreased sharply with increase or decrease in temperature (Fig. 2, left). In other trials, conidia germinated at temperatures as low as 2° C. and, in the greenhouse, at a temperature as high as 25° C. However, germination was poor at these extremes.

Chilling the spores for a short period prior to placing them at different temperatures increased germination. Conidia were collected in a vial and placed at 12° C. for 1½ hours. Examinations at this time showed no germination or even swelling of the conidia. Hanging drop slides were then made and put at temperatures of 4° to 24° C. The results were as follows:

Germination temperature (°C.)	Percentage Germination of Spores: Not Chilled	Chilled
4	9.5	16.7
8	11.6	20.5
12	30.2	21.0
16	19.7	22.6
20	<2.0	22.3
24	0.0	21.1

Not only did the chilled conidia germinate at higher temperatures, but germination was about the same at all temperatures.

b) *Light* Hanging drop slides of conidia were arranged in a 12° C. temperature chamber so that light from a 3-cell flashlight fell on them while others were so arranged that they were in the dark. They were exposed for 48 hours and batteries were replaced as needed. In 1 experiment, germination was 15.6 per cent in the light and 13.2 per cent in the dark, while in another experiment germination was 31.5 per cent in the light and 31.2 per cent in the dark. Results show that the spores germinate equally well in light or dark.

c) *Maturity of Conidia* To test the effect of maturity upon spore germination, conidia were collected from pustules which had reached different stages of maturity. In addition, a sample of conidia was collected that had fallen within a period of 24 hours as well as a sample that had fallen over a period of a week. The results of the germination trials were as follows:

Conidial Development	*Germination Percentage*
From very young, unopened pustules	0.0
From young, unopened pustules	3.2
From pustules, just opened	16.6
Conidia, just fallen	24.6
Conidia, old, fallen	0.0

Thus, maturity of the conidia is apparently an important factor in their germination. Parallel greenhouse inoculations verified these results. Conidia that had just fallen produced better infections than conidia at any other stage of development.

d) *Moisture Content of Conidia* The fact that newly-fallen conidia consistently germinated better than conidia from pustules just beginning to open suggested that possibly moisture content of the spores is an important factor in germination. Since the effect of spore maturation is difficult to separate from the effect of moisture content, the following indirect approach was made.

Leaves having a large number of pustules just beginning to open were collected. The petioles were removed, the leaves were weighed and allowed to dry on a table in the laboratory and, at stated intervals, were reweighed and the conidia collected. Hanging drop slides were put in a 12° C. temperature chamber. As a check, conidia were collected from leaves that were intact on the same plants and from pustules in the same apparent stage of development. Germination of conidia from control leaves ranged from less than 1 per cent to 7 per cent. At the end of the experiment the leaves were oven dried at 100° C. to a constant weight so that the total amount of moisture and percentages of water loss could be determined. An individual experiment usually required 2 to 3 hours of drying to effect maximum germination.

Results (Figure 2, right) show that with an increased water loss from the leaves, there was an increase in the germination percentage up to a certain point, after which the germination dropped considerably and in some experiments stopped. Germination of conidia from desiccated leaves reached 30 to 40 per cent while that of conidia from control leaves was 1 to 7 per cent. The amount of desiccation needed to increase germination varied considerably from leaf to leaf. This was expected because the initial stage of development and natural desiccation vary from leaf to leaf. This finding agreed somewhat with work of Napper (3) who reported that conidia of *Albugo candida* (Pers. ex Chev.) Kuntze had to lose approximately 30 per cent of their moisture content before they would germinate. It did not agree with her results, however, in that a specific amount of water loss was required.

Another indication that water loss of the leaves affects germination came from checking the germination of conidia from wilted plants. Conidia collected from plants that had not been watered and were wilted germinated much better than conidia collected from comparable turgid plants.

Confirming observations were made in the field. Inoculations were made in outside cold frames and in field plots at Madison throughout the summers of 1950 and 1951. After the disease was established, serial plantings of spinach were made throughout the summer to which the disease could spread by natural means. It was observed repeatedly that, in spite of abundant inoculum, very little spread occurred during periods of rainfall and high humidity. On the contrary, abundant spread occurred during periods of relatively warm, dry days but with nights cool enough for dew formation.

e) *pH* To check the effect of pH on germination, double-distilled water was changed to different levels of acidity and basicity by adding small amounts of dilute HCl or NaOH, respectively. The pH range extended from 3.0 to 11.0 and tests were made at intervals of 0.5. The pH was determined by means of a model H2 Beckman pH-meter.

Addition of conidia to the solutions at the different pH levels immediately changed the pH. To overcome this, weak buffer solutions were added to the solutions. However, the concentration required to maintain the pH inhibited germination, whereas the concentrations which did not inhibit germination were too weak to maintain the pH. Because an accurate determination of the effect of pH seemed too difficult, a method giv-

TABLE 1.—*Effect of pH on germination of conidia of Albugo occidentalis and effect of germination on the pH*

Original pH	pH after 24 hours	Germination percentage
3.0	3.0	Trace
3.5	3.8	7.8
4.0	5.2	19.9
4.5	5.7	22.3
5.0	5.8	22.8
Ck. 6.2 [a]	5.8	40.1
7.0	6.0	39.4
8.0	6.5	36.0
9.0	6.2	26.8
9.5	6.1	19.4
10.0	6.7	19.9
10.5	9.2	7.1
11.0	10.7	5.1

[a] Double distilled water with no adjustment of pH.

ing an indication of the pH effect was used in which the conidia were collected in double-distilled water and the pH then adjusted. At the time the germination counts were made, the pH was again measured.

Results (Table 1) show that germination in influenced by the initial pH but that as the conidia germinate they change the pH. At the extremes there is little change, but at the other pH levels the change is marked and tends toward neutrality or slight acidity. Although the conidia germinated over a wide range, germination was poor at the extremes and the zoospores that were formed failed to develop germ tubes.

Factors Affecting Disease Development.—The speed with which symptoms develop depends primarily on the air temperature to which the inoculated plants are subjected. The disease develops more rapidly in plants at 28° C. than at 16° C. Cool temperatures, however, are more favorable for the development of conidia. With a decrease in temperature there is an increase in the amount of conidia produced, and as the fungus develops more pustules are formed. In addition, the pustules formed at cool temperatures are larger than those formed at the warmer temperatures.

The effect of temperature on oospore production is more pronounced than on conidial production. Oospores are produced more rapidly and abundantly at warm temperatures than at cool temperatures (Fig. 1, C-D). At 28° C. the initial production of conidia may be fairly abundant, but conidial production very rapidly becomes suppressed and oospore production is profuse.

The maturity of the plant also influences the rate at which symptoms develop. Conidia are produced only slightly more rapidly in seed plants than in vegetative plants. Oospore production, however, is much more rapid and abundant in seed plants. This is accentuated by the temperature so that at 28° C. oospore production is visible a few days after symptoms appear and, as the disease develops, large numbers of oospores are produced while conidial production is suppressed and may be stopped.

Plant vigor also affects development of the disease. Healthy, vigorous plants were easiest to infect and in them the fungus developed more rapidly. Plants infected with Cucumber virus 1 were difficult to infect, and when they did become infected, the fungus tended to remain localized.

Growing plants at different temperatures for a week to 10 days prior to inoculation had little effect on infection and disease development. However, if plants were grown at warm temperatures until they became tall, chlorotic and spindly, they were difficult to infect.

DISCUSSION.—The germination of conidia of species of *Albugo* has been an objective of experimenters for a long time. Numerous investigators have encountered difficulties in setting up conditions which would result in dependable germination of conidia, and many ideas have been advanced to explain the difficulties. Eberhardt (1), working with *A. candida*, believed that maturity of the conidia was the most important factor. He found that conidia from pustules just ready to open germinated best. Also working with *A. candida*, Melhus (2), believed that because the conidia are basipetally formed and are borne in a pustule, precautions for obtaining mature spores were not necessary. He believed that temperature was the most important factor in germination. He found that the conidia germinated at 0° to 25° C. and, although the optimum was not definitely determined, he believed it to be near 10° C. Napper (3), working with *A. candida*, also encountered trouble in spore germination. Her experiments led her to conclude that it was necessary for the conidia to undergo a loss in moisture content before they would germinate. She found that if the conidia lost about 30 per cent of their total moisture, they germinated equally well between 1 and 18° C.

In addition, many experimenters found that the rate at which the conidia germinate depends on the season. While working with *Albugo* spp. Zalewski (7) found that in summer, germination occurred in 2 to 3 hours, while in the fall 1 to 3 days was required. Melhus and Napper found that the conidia of *A. candida* also germinated faster in summer than in other seasons.

In the present studies with *A. occidentalis*, several factors were found to affect conidial germination. Conidia from pustules just opening germinated much better than those from pustules in any other stage of development. Freshly-fallen conidia showed the best germination while conidia that had been shed from the pustules for several days did not germinate. There was a distinct temperature optimum for germination at about 12° C. A certain degree of desiccation also caused a marked increase in the percentage of germination. Light and the season of the year had no important effect on the amount of germination. Germination occurred over a wide pH range with an optimum near the neutral point.

Results obtained relative to germination have a significant bearing on the geographical range of the

spinach white rust disease. Although the disease has been introduced to many sections of the country on fresh market shipments from Texas it has never established itself outside the extreme southwest, except for a limited extension to the Arkansas River Valley of eastern Oklahoma and western Arkansas. The Winter Garden area of Texas, where it is most severe, is a semi-arid area with low humidity. During winter the days are relatively warm and the nights are cool with heavy dew formation. Thus, warm days and low humidity effect desiccation of conidia while the moisture condensation and cool temperatures of the nights provide the other necessary factors for germination and host penetration. Since germination and penetration occur in a time as short as 2 to 3 hours, the conditions so often present in southwest Texas are ideal for a rapid increase in the disease.

The disease occurs in the lower Arkansas River Valley, but epidemics are local and sporadic, due to the fact that periods of suitable conditions for spore germination are sporadic in occurrence. Spinach is grown during a period when the area receives considerable rainfall and periods of relatively dry weather are sporadic. This effect is substantiated by observations made at Madison during the summers of 1950 and 1951. In experimental spinach plantings in field and outside cold frames, white rust did not spread and develop during periods of wet, cloudy weather, but rather in periods of clear, relatively warm dry days and cool nights.

Air temperature and host maturity were found to have a marked effect on the development of the disease following inoculation. In vegetative plants the disease is rarely systemic and the symptoms are confined to the leaves and petioles. However, on seed plants, systemic invasion is common and both conidia and oospores may be produced on any above-ground part of the plant. Although infected parts are sometimes twisted, the hypertrophy and hyperplasia common to some white rust diseases are not present. In greenhouse studies it was found that the disease develops more rapidly in warm temperatures. More conidia are produced at cool temperatures while oospores are abundantly produced at warm temperatures. In addition, many more oospores and fewer conidia are produced on seed plants. This is augmented by the temperature so that in a seed plant at 28° C., oospore production starts almost immediately after the symptoms appear while few or no conidia are produced.

This leads to a very important control measure in the field. When the harvest season is over the remaining plants are usually left in the fields. As the temperatures and day lengths increase, these plants bolt to produce seed stalks. If the plants are infected, the increased temperature along with the maturing of the plant will favor development of oospores in extreme amounts. However, if the crop residue is promptly plowed under after harvest, this potential source of oospores is destroyed and the amount of inoculum in the field is greatly reduced.

SUMMARY

The effects of environmental factors have been studied with respect to the initiation and development of the white rust disease of spinach.

All commercial varieties of spinach and 89 foreign introductions were found to be susceptible, as well as *Chenopodium capitatum*.

The disease develops more rapidly at warm temperatures, but more development and spread occur at cool temperatures. Conidial production is suppressed at warm temperatures and is very profuse at cool temperatures, while oospore production is very abundant at warm temperatures.

Oospores are formed much more abundantly on seed plants than on vegetative plants.

In laboratory studies of conidial germination, best results were obtained when double-distilled water was used as the germination medium. Light had no effect upon germination. The length of time for germination to take place was not influenced by the time of year. Within limits, the pH was found to have little effect on germination of the conidia. The maturity of conidia is a factor in their germination and conidia from pustules just beginning to open germinated better than those from any other stage of pustule development. The temperature range in which germination was found to take place extended from 2° to 25° C. with an optimum near 12° C. With an increased amount of water loss from infected leaves, germination increased until a point was reached beyond which little or no germination took place. The amount of water loss giving the best germination varied from plant to plant.

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DEVELOPMENT OF WHEAT HEADBLIGHT INCITED BY HELMINTHOSPORIUM SATIVUM[1]

Axel L. Andersen [2]

Helminthosporium sativum P.K. and B. was first described by Pammel, King and Bakke (11) as the cause of a new disease of barley. Its importance became evident approximately a decade later when Christensen (2, 3) and Dosdall (5) published extensive investigations on the pathogen. Recently, Machacek and Greaney (10) have reviewed the principal literature dealing with *H. sativum*.

The importance of *Helminthosporium sativum* as a headblight pathogen is reflected in the losses to wheat and other cereals through reduction in quality of the grain due to discoloration of kernels (8, 10), through direct lowering of yield from shrivelling of the grain, or, as a result of planting infected seed, through impairment of germination, seedling emergence, and yield of the succeeding crop (7, 8).

The purpose of this study was to determine more specifically the effects of air temperature on growth, sporulation, and spore germination of *Helminthosporium sativum*, and the relation of stage of head maturity, air temperature, and air moisture to disease development.

MATERIALS AND METHODS.—Cultures of *Helminthosporium sativum* were obtained from Dr. J. J. Christensen, University of Minnesota; single spore isolates were used. Materials and methods were identical with those previously reported (1) with few exceptions. Bacto bean pod agar was the standard medium for studies of growth and sporulation as well as for producing the conidia used as inoculum. For studies on growth, agar in Petri plates was centrally seeded with a few conidia.

A standard inoculum dosage of 1 million conidia in water suspension was applied by atomizer to the heads in each pot of 10 wheat plants. After inoculation the plants were exposed to continued wetness in the inoculation chambers. The percentage of infected spikelets (amount of infection) and the percentage of killed spikelets (disease severity) were used as bases of comparison in the pathological studies.

Newthatch spring wheat was the principal variety used in the greenhouse experiments. Four stages of development were used: 1) before-flowering, heads emerged from the boot, but no extruded anthers present; 2) flowering, flowers in the process of anthesis; 3) after-flowering, small immature seeds present; and 4) past-flowering, kernels were nearly filled out and in the milk-to-soft-dough stage.

EXPERIMENTAL RESULTS.—*The Relation of Temperature to Growth, Sporulation, and Spore Germination in Vitro.*—The minimum, optimum, and maximum temperatures for growth of the *Helminthosporium sativum* isolate used for plant infection studies were 0-4° C., 24-28°C., and 35-39°C., respectively; they agree with those found by previous workers (5, 9, 12). In comparison, few or no conidia were produced at or below 16°C. and at 32°C. or higher. Good sporulation resulted at 20°, 24° and 28°C., with the highest initial number produced at 28°C.

Rate of spore germination and length of germ tubes provided ideal measures of fungus development at various temperatures. The most rapid germination occurred at 32°C. At this temperature, 96 per cent of the conidia had germinated in 3 hours and the germ tubes were as long as the conidia. At 28°C., 69 per cent of the conidia were germinated and the germ tubes were ½ the length of the conidia. Short and knobby germ tubes were evident on approximately 50 per cent of the conidia incubated at 24° and 36° C. A different response was noted 2 hours later. At this time, all the conidia incubated at 28° and 32°C. had germinated and nearly all at 24° and 36°C. had germinated. At this time, however, the germ tubes on the conidia incubated at 28°C. were more than 3 times as long as those produced at 32° C., and considerably longer than those at 24°C. Thus the rate of growth of the germ tubes after 5 hours incubation appeared to follow very much the same curve as that for colony growth, with the optimum temperature at 28°C. and some growth occurring at 36°C.

Relation of Head Maturity to Disease Development.—There was every indication in initial experiments with Helminthosporium headblight that susceptibility to infection is determined by the stage of head maturity at the time of inoculation. Records of individual spikelets on wheat heads in various stages of development from the before-flowering to the past-flowering stages indicated that a high degree of resistance was maintained up to the time of anthesis. After flowering the spikelets became susceptible.

Susceptibility increased as plants passed from the flowering to the past-flowering stage of development. This was demonstrated in an experiment in which inoculated and noninoculated wheat was exposed for 36, 48 and 60 hours at 20°, 25° and 30°C. in the humidity tents. Since comparable reactions were obtained at the 3 exposures, only the results after 36-hour exposure are illustrated in Fig. 1.

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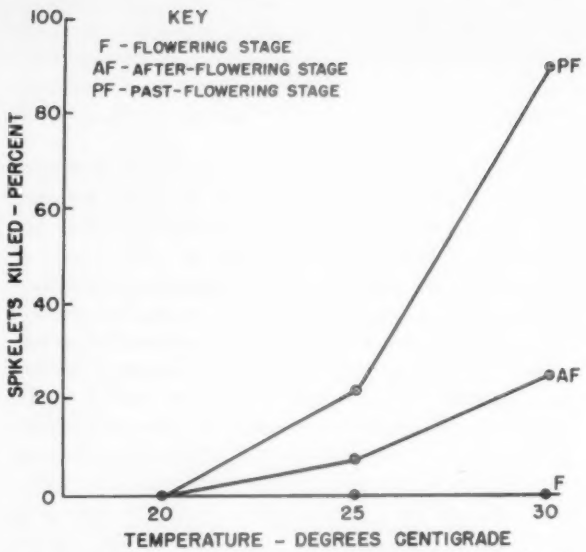

FIG. 1. The comparative susceptibility of Newthatch wheat in different stages of development to infection by *Helminthosporium sativum*. Plants were exposed to continued wetness for 36 hours after inoculation.

Plants inoculated in various stages of development differed in symptom expression. Those inoculated during the flowering stage produced dark brown to creosote-colored lesions on glumes, lemma, palea, and rachis, and the lesion symptoms persisted after death of the plant parts, giving them a mottled brown appearance. On the other hand, the heads inoculated in the past-flowering stage of development were very rarely discolored; the spikelets rapidly lost their green color, becoming bleached and straw-colored as soon as they were killed.

TABLE 1.—*Helminthosporium headblight resulting from exposure of plants, inoculated in the past-flowering stage, to different periods of continued wetness at different temperatures*

Temp. °C.	Exposure period	Spikelets	
		Infected [b]	Killed [c]
	Hours	Per cent	Per cent
15	12	0	0
	24	0	0
	36	0	0
	48	2	0
20	12	0	0
	24	6	0
	36 [a]	18	0
	48	61	31
25	12	Trace	0
	24	12	4
	36	73	8
	48	97	40
30	12	3	0
	24	16	24
	36	84	22
	48	99	86

[a] Except for this treatment, data are based on 23 to 30 heads, involving 200-250 spikelets.
[b] Recorded 6 days after inoculation.
[c] Recorded 13 days after inoculation.

TABLE 2.—*Helminthosporium headblight resulting from exposure of plants, inoculated in the flowering stage, to different periods of continued wetness at different temperatures.* [a]

Temp. °C.	Exposure period	Spikelets		
		Infected [b]	Killed	Corrected [c] wt. of seeds
	Hours	per cent	per cent	gm.
15	12	0	0	1.5
	24	0	0	1.5
	36	0	0	1.8
	48	7	0	1.3
	60	11	0	1.7
	72	17	0	1.3
20	12	0	0	1.5
	24	7	0	1.0
	36	77	0	1.2
	48	100	0	0.7
	60	100	0	0.6
	72	100	16	0.2
25	12	22	0	1.4
	24	100	0	0.9
	36	100	28	0.2
	48	100	38	.1
	60	100	79	.1
	72	100	100	0.1
30	12	39	0	1.2
	24	82	0	0.8
	36	100	14	0.3
	48	100	37	0.2
	60	100	88	0.1
	72	100	97	0.1

[a] Data based on 21-31 heads, involving from 214-342 spikelets.
[b] Recorded 7 days after inoculation.
[c] L.S.D. at 5 per cent level = 1.03. Wt. of seed corrected on the basis of the total number of spikelets, using the formula:

$$\frac{\text{Total wt. of seed per pot} \times 100}{\text{Total No. of spikelets}}$$

The size of the kernel progressively increased with the stage of host development at time of inoculation, but all were shrunken and dark in color. Many infected kernels from plants inoculated in the past-flowering stage showed the characteristic "black point" symptom. Those kernels from plants inoculated in earlier stages of plant development possessed small black or brown lesions. Heavy infection during the flowering and after-flowering stages resulted in a lowered yield whereas infection in the late stages of head maturity affected quality only.

Effect of Air Temperature and Moisture on Disease Development.—Several experiments were conducted under controlled conditions on the effect of exposure of plants, inoculated in the flowering, after-flowering, and past-flowering stages of development, to different periods of continued wetness at 15°, 20°, 25° and 30° C. (Table 1, 2, and Fig. 1). Although the data varied a little in each experiment, the general trend was the same. These results, together with those from other

experiments, indicated a wide range of temperature favorable for infection of wheat by *Helminthosporium sativum*, and for subsequent development of the disease, with the optimum probably between 25° and 30°C. These conclusions agree with the observations of other workers (3, 4, 6, 7, 8) who noted that warm, humid weather favored the development of *H. sativum*.

There is the possibility that the stage of plant development may be responsible for differences in disease severity at 25° and 30°C. Those plants inoculated in the past-flowering stage of development nearly always developed more severe headblight at 30°C. then at 25°C. (Table 1). Those plants inoculated in the flowering stage appeared to develop equally severe headblight at 25° and 30°C. (Table 2.)

Disease development was less severe on plants exposed to continued wetness at lower temperatures. Yield was not reduced and no spikelets were killed on plants inoculated in the flowering stage and exposed at 15°C. Many spikelets became infected, but few were killed at 20° C. with 36 or 48 hours exposure. A 72-hour exposure at 20° C. was required to obtain killing comparable to that for 36 hours exposure at 25° and 30°C.

There appeared to be a different minimum period of exposure to continued wetness at each temperature for maximum increase in infection and, also, a different minimum for maximum increase in disease severity (Table 2). On the basis of yield, the critical time for increase in disease severity was that exposure period resulting in the first killed spikelets. These periods were from 60-72 hours at 20°C., and 24-36 hours at 25° and 30°C. for wheat heads infected during the flowering stages.

Symptom Development.—Comparisons were made to determine whether the period necessary for development of visible symptoms (incubation period) was influenced by different periods of exposure at different temperatures. The shortest incubation period (1½ days) was with those plants exposed for 36 hours at 25° and 30°C. Exposures of less than 36 hours at these temperatures resulted in correspondingly longer incubation periods. For example, with a 12-hour exposure the incubation period was 4 days. Incubation periods were longer for plants exposed at lower temperatures. For example, plants inoculated in the flowering stage and exposed for 48 or 60 hours at 20°C. had an incubation period of 2½ days as compared to 7 days at 15°C. It is evident that period of exposure and temperature at the time of exposure were both important in determining the length of the incubation period.

Symptom Expression.—Disease symptoms varied with the periods of exposure to moisture and temperature. In daily observations, coloration was most pronounced on those plants in which infection developed the least rapidly. For example, on plants exposed for 60 to 72 hours at 20°C., the infected portions became dark to reddish-brown, and when the heads were finally killed, the dark discoloration was retained to a certain degree, producing a dark mottled to bleached appearance. Exposures at temperatures more favorable for infection (25° and 30° C.) for 48 hours or more resulted in rapid killing of the wheat heads and little development of color.

Sporulation.—Influence of temperature and moisture on extent of sporulation on wheat heads was demonstrated in 2 series of experiments using humidity tents as incubators. Each humidity chamber measured 4′ × 8′ × 4′ high. The chamber was formed by covering a metal frame with a layer of muslin which was kept moist by an over-head atomizer. A second metal frame covered with muslin was placed over the first frame to reduce the effect of the greenhouse temperature upon the temperatures inside the chamber. Humidity in the chamber was maintained by a Standard Humidifier. Temperatures inside the tents were controlled within ± 0.5 C. by thermostatically controlling the temperature of the water passing through the humidifier and the over-head atomizers. First, infected plants with well developed symptoms of Helminthosporium headblight were placed in the humidity tents and exposed for several days at 15°, 20°, 25°, and 30° C. Wheat heads were removed at 24-hour intervals and the number of conidia determined. Conidia were produced on the diseased heads within 24 hours after the plants were first wetted at 25° C. After 48 hours exposure, conidia were present on all the heads with maximum sporulation occurring at 25° C. In the next experiment on sporulation the plants were inoculated and exposed at 20°, 25° and 30°C. in the humidity tents. Wheat heads were removed for examination at 24-hour intervals from the time of inoculation. Results from 3 of these experiments were practically identical in that the first new spores were formed 4-5 days after inoculation at 25° and 30°C. Although the wheat heads were abundantly infected, relatively few conidia were produced even after 6 days exposure at 25° and 30°C. Further studies were made in which infected heads were removed and placed in small humidity chambers maintained at different temperatures from 4° to 32° C. Four heads were examined each day over a 6-day period. No conidia were produced on the heads incubated below 16° and above 28°C. These results agree closely with those obtained on the effect of temperature on sporulation on bean pod agar.

Discussion.—When inoculated plants were exposed to continued wetness at temperatures of 20°, 25° and 30°C. there was generally a definite period during which a considerable increase in infection occurred and another period during which disease severity markedly increased. For example, infection increased on plants inoculated in the flowering stage and incubated at 25° C. from 22 per cent with a 12-hour exposure to 100 per cent with a 24-hour exposure; disease severity increased from 38 per cent with a 48-hour exposure to 79 per cent with a 60-hour exposure. These exposure periods were noted in all the experiments on Helminthosporium headblight. In this respect, *Helminthosporium sativum* differed from *Gib-*

berella zeae (1). For the former pathogen the exposure period most favorable for infection was not the most favorable for disease development; for *G. zeae* these periods were the same. Once the symptoms of spikelet infection by *G. zeae* became evident, that spikelet was invariably killed. With *H. sativum* the shortest period of exposure after inoculation necessary for heavy infection did not result in either killing of the spikelets or in a significant reduction in yield. It was only after prolonged exposure that yield reduction occurred and spikelet killing became evident.

Under conditions of high inoculum potential, the environment would be the controlling factor in the establishment of infection and subsequent development of the disease. For example, if 25°C. air temperatures prevail, it would be expected that 12 hour periods of moisture resulting from heavy dews, small showers, etc., would be followed shortly by heavy field infection with *Helminthosporium sativum*. The disease would not be expected to increase unless similar conditions occurred frequently during the heading period. However, if the initial moist period had lasted for a longer period (24-36 hours), a heavy loss in yield would be expected even though there might not be any apparent killing of the individual spikelets or heads. The killing would appear only if weather conditions favorable for infection had been present for at least 60 hours during the heading stage. With lower air temperatures, correspondingly longer periods of moisture would be required to produce the same results. On the other hand, as the plants neared maturity, an increasingly greater amount of infection would be expected under favorable environmental conditions because of increased host susceptibility. This does not imply that correspondingly greater reductions in yield would accompany infection. Kernels would already be well developed and any infection in the later stages would result in a discoloration or shrivelling of the seed only.

SUMMARY

Growth of *Helminthosporium sativum* mycelium and germination of the conidia occurred at temperatures from 4° to 36°C. with the most rapid growth of the mycelium and elongation of the germ tubes occurring at 28°C.

The greatest sporulation occurred at 24-28°C. No conidia were produced below 16° or above 28°C. on agar or infected wheat heads.

Little infection occurred on wheat heads inoculated prior to flowering. From the flowering to the past-flowering stages, wheat increased in susceptibility to Helminthosporium headblight.

Temperatures from 25 to 30°C. were the most favorable for infection and headblight development. At these temperatures, exposure to continued wetness for 12 hours resulted in plant infection, whereas a 36-hour exposure was required for killing of the spikelets.

Visible symptoms were produced within 1½ days after inoculation on plants exposed for 36 hours to continued wetness at 25° to 30° C. Longer periods were necessary for the development of symptoms at the lower temperatures and with shorter periods of exposure to continued wetness.

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THE RELATION BETWEEN FUNGITOXICITY, PERMEATION, AND LIPID SOLUBILITY [1]

Saul Rich and James G. Horsfall

For a toxicant to be effective it must be not only inherently poisonous, but also able to reach its site of action. Most toxicants must be able to penetrate the cell. An analogous example is the "shaped-charge" for artillery shells. Two missiles might contain the same amount of explosive and hence have the same potential power. That with the properly shaped charge, however, can penetrate a much thicker barrier of armor than the other. This paper will attempt to explore what constitutes a "shaped-charge" in fungitoxicants and how it can be appraised. Specifically, what changes may be made to the fungitoxic molecule that will enhance its ability to enter the spore and how we can tell whether the change has also changed the inherent toxicity as well?

First of all, information was needed to distinguish the effect of molecular changes on inherent toxicity from their effect on permeation. It seemed possible that the standard dosage-response curve could provide this information.

Underlying the use of dosage-response curves is the basic principle that individual organisms of the same species vary not only in physical dimensions but also in their response to different chemical treatments. Moreover, the range of variation usually has a different magnitude for different characteristics of the same population of organisms. Furthermore, the range of variation is constant for a single characteristic of 1 population.

These principles apply to spore toxicity experiments (15). In practice, spore toxicity data are obtained by subjecting aliquots of a spore population to a graded series of dosages. Data are recorded as per cent inhibition of spore germination at each dosage. Data so acquired usually generate a straight line, when the log of the dose on the X axis is plotted against equalized standard deviation units on the Y axis. These equalized standard deviation units, or probability units, are called probits (3). The range in variation of the response of the population[2] is expressed by the range of dosage required to measure 1 probit of response. Therefore the slope of the resulting line is governed by the range of variation. If 1 probit of response is measured by a large range in dosage, the slope is flat. Conversely if 1 probit is measured by a narrow range of dosage, the slope is steep.

Since the range of variation reflects the action of a toxicant on a vital process or processes in a spore population, and since slope is delimited by this range, slope may be assumed to be governed by mode of action. On the basis of these assumptions, it may be postulated that if 2 toxicants are acting by 2 different pathways, the responses of the biological population will have distinctly different standard deviations, and hence non-parallel slopes. Further, a population of spores treated with compounds acting by the same toxic mechanism should exhibit the same variation and the same slopes.

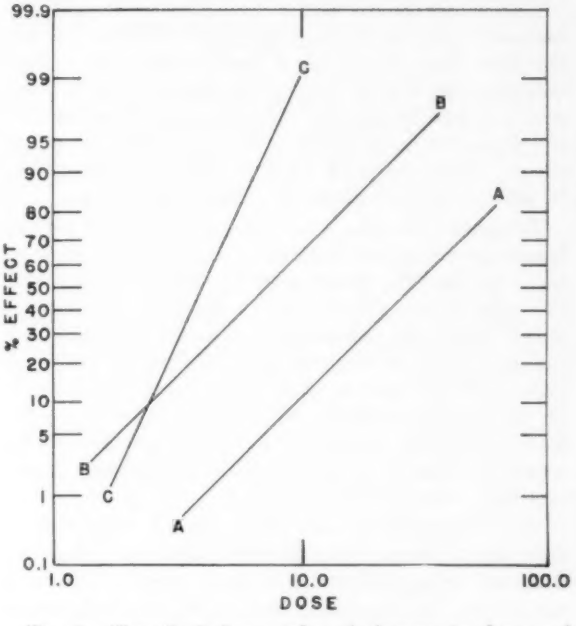

FIG. 1. Hypothetical examples of changes in slope and ED 50. Compounds B and C are derivatives of Compound A.

In making these comparative measurements of slope,[3] all other factors should be kept as nearly equal as possible. There is the chance, of course, that 2 toxicants would coincidentally result in parallel slopes, even though acting by different mechanisms. This is probably remote, especially if the compounds concerned are chemically related.

The transformed dosage-response curve may be characterized by its position as well as its slope. The position of the slope is usually expressed as the dosage required to affect a given proportion of the population: usually 50 per cent. This point is referred to as ED 50 (the dose for 50 per cent effectiveness), also known as LD 50. The less material required at ED 50, the more potent the material is considered to be.

Potency is actually determined by 1) mechanism of action, and 2) ability of the toxicant to reach its site of activity. If 2 materials have the same mechanism of

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[2] This is the range of variation for the whole population over a graded series of dosages, and not the variation of repeated aliquots at a single dose.

[3] The numerical expression of slope as used in this paper, $\frac{1}{\text{Log ED 84} - \text{Log ED 50}}$ is derived from Gaddum (6).

toxic action, then the difference in their potency must be attributable to difference in their ability to penetrate. How information on slope and ED 50 may be used to distinguish permeation from inherent toxicity is illustrated by the hypothetical examples in Fig. 1.

Compounds B and C are derivatives of A. All are assumed to be in the same physical condition. B has the same slope as A, but is much more active. This indicates that B may be more permeative than A. Compound C has an entirely different slope from both A and B; hence, is probably acting by a different mechanism. Since the slope for C is different, nothing can be said for certain about its relative permeation.

To check our theory, we needed a series of related fungitoxicants which differed in their ability to penetrate spores. The Overton-Meyer solvent theory (10, 11) offered the clue as to the types of substituents which would aid penetration. This theory states that the penetration of organic material through the lipid-water colloidal system of the cell membrane depends on the partial solubility of the material in both the water and fatty phases. The Overton-Meyer theory postulates that fat solubility is relatively more important than water solubility.

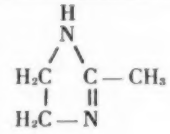
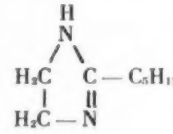
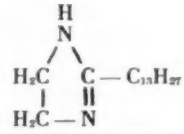
Series of fungitoxicants were needed which would have increasingly lipophilic substituents. The imidazolines (glyoxalidines) appeared to be suitable as a test series. Wellman and McCallan (14) had shown that the potency of homologues of 2-imidazoline increased with the length of aliphatic side chain. If this were caused by improvement in penetration, then the slopes should be parallel.

Dr. R. H. Wellman kindly provided samples of 2-methyl-, 2-amyl-, and 2-tridecyl-2-imidazoline, containing, respectively, 1, 5, and 13 carbons in a side chain in the 2-position. The compounds were assayed with spores of *Stemphylium* (previously called *Macrosporium*) *sarcinaeforme*. Care was taken to test all 3 on the same population of spores. Different populations of spores might show different standard deviations and hence different slopes.

Data in Table 1 were obtained with the depression-slide technique described by Barratt and Horsfall (2). Data on potency confirm those of Wellman and McCallan (14). Potency increases rapidly with length of side chan. From data it is further deduced that the slopes for 3 selected homologues of 2-imidazoline are essentially parallel, and hence the huge differences in potency are related to permeation and not to differences in toxic mechanism. The slopes are considered parallel even though they vary from 9.37 to 11.01, because in this range it takes only an extremely slight difference in slope to result in a very large change in the numerical representation. These differences therefore are probably not significant.

Because of their emulsifying action, and because we could not analyze for imidazolines, the relative oil-water solubilities of this series could not be determined. We therefore turned to the nitrosopyrazoles, which were also suited for testing our hypothesis, and

TABLE 1.—*Dosage response data resulting from fungitoxicity tests of imidazoline homologues against Stemphylium sarcinaeforme*

Compound	Structure	ED 50 in micromoles per sq. cm.	Slope
2-Methyl-2-imidazoline		3.57	9.37
2-Amyl-2-imidazoline		0.70	11.01
2-Tridecyl-2-imidazoline		0.0037	9.97

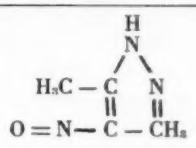
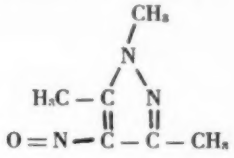
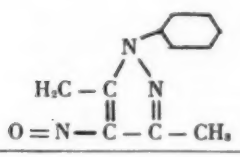
in addition gave colored solutions. Hence, their solubilities could be determined colorimetrically.

McNew and Sundholm (9) had reported that the potency of the nitrosopyrazoles increased with the size of the hydrocarbon side group. The 3 nitrosopyrazoles which we tested were furnished to us by Dr. H. D. Tate of the Naugatuck Chemical Co., and differed only by the size of the hydrocarbon substituent in the 1-position. Dosage-response data[4], water solubility, and oil-water partition coefficients were obtained for these nitrosopyrazole derivatives. The oil-water partition coefficient indicates the relative amounts of the compound that will dissolve into oil and water when the compound is shaken up with a mixture of the two. In other words, it is a relative measure of how much should get through a membrane made up of lipids and water. Olive oil was used on the assumption that it approximates the glycerides occurring in the membranes of the spore.

Data in Table 2 agree with those of McNew and Sundholm that potency increases with the size of the substituent carbon group. They show further that the slopes are parallel for the 3 nitrosopyrazole derivatives. Here again we deduce that differences in fungitoxicity are due to differences in permeation. The oil-water partition coefficients are directly related to potency. Water solubility, on the other hand, is inversely related to potency. The relation of oil-water partition coefficient to the fungitoxicity of the nitrosopyrazole derivatives is graphically presented in Figure 2. On the vertical axis potency is expressed as the reciprocal of ED 50. The reciprocal is used be-

[4] Obtained by using the laboratory sprayer technique described by Barratt and Horsfall (2). The organism was *Stemphylium sarcinaeforme*.

TABLE 2.—*Dosage-response data, water solubilities, and oil/water partition coefficients for derivatives of 4-nitrosopyrazole. The dosage-response data were obtained using the spores of Stemphylium sarcinaeforme*

Derivative	Structure	Slope	ED 50 in μ moles/sq. cm.	Water solubility at 25° C. mg/100 cc	Oil/water partition coefficient
3,5-Dimethyl-4-nitrosopyrazole		11.5	1.36	over 200	0.48
1,3,5-Trimethyl-4-nitrosopyrazole		11.5	0.043	187	1.53
1-Phenyl-3,5-dimethyl-4-nitrosopyrazole		11.5	0.00017	27	6.66

cause the more potent the material, the smaller is its ED 50. The points on the graph are indicated by the substituent in the 1-position in the molecule.

DISCUSSION.—The relation of lipid solubility to permeation has been discussed by workers in other fields (1, 4, 7). That this principal would also apply to fungicides is an obvious corollary. More emphasis appears to have been placed on the water solubility of fungicides than on their lipid solubility. This situation undoubtedly arose from the fact that fungicides for plant protection are almost always applied in water. Water solubility, may of course, be of utmost importance in those cases of fungicides acting at the cell surface. For the great number of fungicides which undoubtedly act by penetrating the plasma membrane, the basic requirement for penetration must be met for obtaining optimum effectiveness.

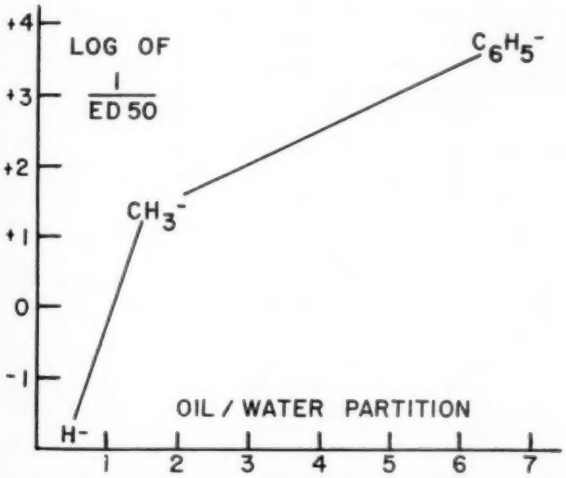


FIG. 2. The relation between oil/water partition and potency of some derivatives of 4-nitrosopyrazole. Potency expressed as the log of the reciprocal of the ED 50. Points on the graph indicated by the substituent in the 1-position of the toxic molecule.

The answer to optimum penetration is not solely lipid-solubility. Homologous series of toxicants exhibit peaks of activity beyond which increasing size of lipophilic side-chain may actually result in diminishing effectiveness. Data of this type are presented by Wellman and McCallan (14) and Tattersfield and Gimingham (13). These peaks can perhaps be explained by the necessity for a proper balance between lipid solubility and water solubility to achieve maximum penetration. This optimum balance will undoubtedly be related to the type of lipids involved. Two different organisms, for example, may contain 2 different lipid complexes. Testing the same homologous series against these 2 different organisms may show that 1 derivative is most active against 1 organism while another derivative is most active against the second organism. The lipids of the 2 organisms may differ so drastically that 1 organism may not be affected by any member of the homologous series. Differences in the lipids of host and parasite may also result in 1 homologue being most phytotoxic while another homologue is most fungitoxic. This may be the explanation of the situation described by Wellman and McCallan (14). In testing derivatives of 1-hydroxyethyl-2-imidazoline, they found that the 2-tridecyl derivative was the most phytotoxic of the series while the 2-heptadecyl derivative was the most fungitoxic.

A study relating solubilities to toxicity cannot be made without considering the partial saturation effect described by Ferguson (5) for non-specific poisons. Ferguson theorized that substances which would pro-

duce their disruptive effect merely by their presence as inert foreign molecules would have the same degree of biological action when applied at the same proportional saturation in a given medium. He reasoned that the partitioning of a toxicant between the phase in which it is applied, e.g., water, and the phase in which it acts, e.g., lipids, would be proportional to the solubility saturation of the phase in which it was applied. Examination of the nitrosopyrazole data reveals that Ferguson's effect is not exhibited by this series. The 2 most obvious inferences which may be drawn are 1) the nitrosopyrazoles act by virtue of an active group, e.g., the nitroso side group, and/or 2) the nitrosopyrazoles do not depend on solubility in lipids for their ultimate activity, but merely for ease in permeation.

Only when dosage-response curves are parallel are they useful in comparisons of permeation. Slope data, in order to be compared, must be obtained under identical environmental conditions. Undoubtedly, there are factors other than differences in mode of action which can cause slopes to be nonparallel. McIntosh (8) described changes in slopes with DDT depending on whether he used the colloidal form or crystalline aggregates. Stringer (12), however, applied a retention correction to McIntosh's dosage-mortality data and showed that in fact the slopes for the 2 physical forms were parallel. Our study makes no attempt to interpret the significance of nonparallel slopes resulting from tests in which all conditions are not reasonably similar.

Comparative dosage-response data may be used to determine if the chemical modification of a toxic molecule has changed 1) the ability of the toxicant to enter the spore or 2) the ability of the toxicant to damage the cell. Once the effect of the molecular change is identified, the modification could be used in the production of more effective compounds. This could not only accelerate the development of new fungitoxicants but should also be useful in research on mechanisms of action.

SUMMARY

It is proposed that dosage-response data be used for differentiating between inherent fungitoxicity and permeation.

Series of fungitoxic compounds were chosen which should increase in ability to permeate into cells by virtue of increasingly lipophilic side-groupings. The choices were made on the basis of the Overton-Meyer theory of cell permeation.

These 2 series, derivatives of 2-imidazoline and of 4-nitrosopyrazole, showed increasing fungitoxicity with increasing size of hydrocarbon side group.

Within the series, the dosage-response slopes were parallel, indicating that there was no change in mechanism of action as the hydrocarbon side group was enlarged.

The 4-nitrosopyrazole derivatives showed increasing fungitoxicity as their oil:water partition coefficients increased. Their water solubilities, however, were inversely related to fungitoxicity.

The peaks in fungitoxicity reported by Wellman and McCallan (14) for the 2-imidazolines, and by other workers for other series, suggest that the most active derivatives must have optimum ratios of lipid solubility to water solubility for maximum penetration.

Cases where 1 derivative is most phytotoxic and another derivative most fungitoxic may arise from decided differences in the nature of the lipids of host and parasite. Differences in lipids may also cause differences in the susceptibility of various fungi to the same toxicant.

The use of dosage-response data to identify the effect of molecular changes on fungitoxicity could be of considerable value in the development of new fungicides.

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Susceptibility of Lycopersicon Species to the Golden Nematode. W. F. Mai. *Lycopersicon esculentum* Mill., the commercial tomato, has been shown to be susceptible to the golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber, by a number of workers. No published record of the susceptibility of other species of the genus Lycopersicon to this nematode has been noted by the author. There are several reports of the susceptibility of Lycopersicon species to the root knot nematode, *Meliodogyne spp.* In greenhouse tests Bailey[1] found that *L. peruvianum* was resistant to attack by the root knot nematode while *L. glandulosum*, *L. hirsutum*, and *L. pimpinellifolium* were susceptible. The resistance of *L. peruvianum* varied with different collections of seed. Several workers have since demonstrated the resistance of certain selections of *L. peruvianum* to the root knot nematode and have obtained crosses between this species and *L. esculentum*. Recently, Taylor and Chitwood[2] have shown that different species of the genus Meloidogyne differ in their pathogenicity to *L. peruvianum*.

In 1948 seeds of several species of the genus Lycopersicon were planted in the greenhouse in soil heavily infested with golden nematode. Young plants were later transplanted to 6-in. pots of heavily infested soil. After the danger of frost had passed the pots were sunk in the soil outside. When the immature females on the roots of the check tomato plants had emerged and had begun to turn from white to cream color, the roots of all plants were harvested and placed in 15 per cent formalin. The number of immature females per gm. of root was estimated for each root system by means of the washed-root technique of Chitwood and Feldmesser.[3]

Lycopersicon peruvianum, *L. glandulosum*, *L. pimpinellifolium*, and *L. hirsutum* proved to be hosts of the golden nematode (Table 1). Two collections of *L. peruvianum* (47B323 and 732) appeared to possess a high degree of resistance. Other collections of this species were more susceptible. The significance of the low immature-female reading obtained on the roots of *L. glandulosum* is doubtful since only 1 plant was tested. *L. hirsutum* was as heavily attacked as *L. esculentum*. The numbers of nematodes on the roots of the different collections of *L. pimpinellifolium* were, in general, intermediate between the numbers found on the roots of *L. peruvianum* and the roots of L. *hirsutum*.

[1] Bailey, D. M. The seedling test method for root knot nematode resistance. Proc. Amer. Soc. Hort. Sci. 38:573-575. 1941.

[2] Taylor, A. L. and B. G. Chitwood. Root knot susceptibility of Lycopersicon peruvianum. Plant Dis. Reptr. 35: 97. 1951.

[3] Chitwood, B. G. and Feldmesser, J. Golden nematode population studies. Proc. Helminth. Soc. Wash. 15: 43-55. 1948.

TABLE 1.—*Results of tests of the susceptibility of several Lycopersicon species to the golden nematode*[a]

Name	Collection No.	No. of Immature Females/ gram of root	No. of Plants Tested
L. peruvianum (L.) Mill.	47B323	0.4	5
L. peruvianum	732	1.0	5
L. glandulosum C. H. Mull.	47B328	2.0	1
L. pimpinellifolium (Jusl.) Mill.	—	5.0	3
L. peruvianum	47B321	8.0	5
L. peruvianum	1028	12.0	5
L. pimpinellifolium	—	16.0	3
L. peruvianum	—	18.0	4
L. pimpinellifolium	45B251	22.0	4
L. peruvianum var. *dentatum*	1246B	29.0	4
L. peruvianum var. *humifusum* C. H. Mull.	1074	33.0	4
L. peruvianum var. *humifusum*	47B403	34.0	4
L. peruvianum var. *dentatum*	47B326	43.0	5
L. esculentum var. Comet	—	51.0	5
L. hirsutum	46B355	76.0	1
L. hirsutum	41B107F	95.0	5
L. esculentum var. Waltham	—	107.0	5

[a] The seeds used in this experiment were obtained through the generosity of Dr. W. S. Porte of the U.S.D.A., Dr. J. M. Watts of the University of Arkansas, Dr. Harold A. Senn of the Canadian Department of Agriculture, and Dr. Bert Lear of the Department of Plant Pathology, Cornell University.

In summary, all species of the genus Lycopersicon tested were found to be susceptible to attack by the golden nematode. The relative susceptibility of the species of this genus to the golden nematode was approximately the same as the relative susceptibility to the root knot nematode as reported in the literature.—Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Physiologic Races of the Tomato Late Blight Fungus.[1] M. E. Gallegly.—A search for resistance to tomato late blight was begun during the summer of 1950 with field plantings of 50 tomato accessions near Huttonsville, West Virginia. During that year West Virginia number 36 (Cherry tomato from North Carolina) remained healthy for 10 days longer than did the other accessions in the plot, although it eventually became severely diseased.

In 1951 field plantings were expanded to include 662 tomato accessions, most of which were obtained from the Plant Introduction Stations at Ames, Iowa and Glenn Dale, Maryland. Late blight was not so severe as in 1950 but disease index readings ranged from very

[1] Published with the approval of the Director of West Virginia Agricultural Experiment Station as Scientific Paper No. 451.

slight to severe. Again number 36 along with several of the other accessions remained healthy for 2 wk. longer than did the majority of the accessions.

Results indicated that there was some resistance in these accessions to the tomato late blight fungus present in the field at Huttonsville, West Virginia. Isolates from different parts of the United States were obtained for artificial inoculations in the greenhouse. WV-2T and WV-6T are West Virginia isolates; M-6 is an isolate from Lancaster, Pa.; M-7 from Indiana; and M-13 from Fort Pierce, Florida. Isolates M-6, M-7 and M-13 were supplied by Dr. Paul Miller. These isolates were cultured on lima bean agar (250 gm. frozen baby lima beans ground for 10 min. by Waring Blendor, 15 gm. agar, and 1,000 ml. distilled water). Inoculations were made by atomizing a zoospore suspension (obtained by incubating sporangia from 7- to 14-day old cultures in distilled water at 12°C for 2 hr.) on 3- 6-wk. old plants. After inoculation the plants were held for 24 hr. in a 20°C. constant temperature room at a 100 per cent relative humidity and 12 hr. day length. The plants were then moved to a 20° greenhouse. After 7 days the plants were examined and placed in one of the following disease index classes: 1) immune or no visible infection; 2) highly resistant plants showing speck and small lesions which do not spread; 3) resistant plants with larger lesions which may spread slowly; 4) susceptible plants with lesions spreading more rapidly but plants not dead; 5) plants dead or dying within 7 days after inoculation.

In a preliminary experiment, numbers 36 and 106 were resistant to isolates M-6, M-7 and WV-6T. An occasional plant of number 36 was susceptible (one count showed 10 resistant and 2 susceptible), indicating that the selection was not homozygous for resistance.

In another experiment, 10 to 15 3-wk. old plants of 11 accessions growing in 3-in. pots of vermiculite were inoculated with each isolate. Results are in Table 1. All the tomato accessions were susceptible to isolates M-13 and WV-2T. Numbers 19, 36, 41, and 731 were resistant to isolates M-7 and WV-6T while only number 36 was resistant to M-6. Number 106 was not included in the experiment due to a shortage of seed.

TABLE 1.—*Disease index readings of 12 tomato accessions inoculated with 5 isolates of Phytophthora infestans*

West Virginia Number[a]	Identity	Disease Index Readings[c] Isolate Number				
		M-6	M-7	WV-6T	M-13	WV-2T
4	49B179	5	4	4	5	5
12	49B331-1	5	5	4	5	5
19	Ornamental Tomato, P_1	4	2	2	5	5
36	Cherry Tomato	2	1	2	5	5
41	Phyllis	4	2	3	5	5
106[b]	PI 108245	3	3	2	5	5
730	1-1 PW	5	4	4	5	5
731	25-1 PAStW	4	2	2	5	5
732	1-5-1 CAPStW	4	5	4	5	5
733	1-5-2 CAPStW	5	5	5	5	5
G-1	From Guatemala	4	4	4	5	5
Break-O-Day		5	5	5	5	5

[a] Numbers 4 and 12 were supplied by W. S. Porte, Beltsville, Md.; numbers 19, 41, 730, 731, 732, and 733 by J. M. Walter, Bradenton, Fla.; number 36 by D. E. Ellis, and W. S. Barham, Raleigh, N. C.; and G-1 by Reiner Bonde, Orono, Maine.

[b] Data from a separate experiment.

[c] See text for disease index classes.

Results show that there are at least 3 races of the tomato late blight organism. Isolates M-7 and WV-6T constitute 1 race, M-6 a second, and M-13 and WV-2T a third when the above varieties are used as hosts. Tomato accessions 36 and 106 are resistant to all available isolates except M-13 and WV-2T.

These data show that despite the fact that there appears to be no resistance to late blight in the common commercial varieties of tomatoes there is resistance in some of the wild types. They also show, as in the potato strain of the fungus, that there are pathogenic races in the tomato strain.—Department of Plant Pathology and Bacteriology, West Virginia University, Morgantown, West Virginia.